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THE HISTOGENESIS OF MYXEDEMA*

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THE existence of an extracellular interfibrillar mucinous substance in the dermis and other connective tissues of patients with a deficiency of thyroid hormone has been well known for many years (1–3). However, it remained for Watson and Pearce (4), in their investigations of localized myxedema, to demonstrate that the extracellular material present in these lesions was composed of the acid polysaccharides, chondroitin-sulfuric acid and hyaluronic acid, mixed with, or in some form of combination with proteins.

That the degree of myxedema, *i.e.*, the amount of mucinous material in the skin, is related to the severity and the duration of hypothyroidism is apparent from clinical observations. However, it has not been possible hitherto to ascertain directly the length of time required for development of the cutaneous changes, nor to follow the disappearance of the myxedema following the administration of desiccated thyroid.

The study of serial biopsy specimens obtained with an electrically driven biopsy punch (5) has made investigation of these problems possible. The technique enables one to take repeated specimens of the skin without local anesthesia and without discomfort to the patient, thus eliminating the artefacts of tissue distortion due to the injection of an anesthetic. All sections can be derived from the same area of skin, so that difficulties in interpretation due to regional variations in the anatomy of the skin are minimized.

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METHODS

Punch biopsy specimens, 3 or 5 mm. in diameter, were obtained by means of an electrically driven hand drill equipped with bits (5). The biopsies were performed at intervals ranging from three days to two months. In most of the patients studied, weekly specimens were secured. At first, several sites were used, but it was found that the lateral aspect of the thigh was most satisfactory, and this was then made the standard site for biopsy. Comparative studies were made only upon sections obtained from the same area of skin. The tissues were fixed in alcoholic formalin (Davidson's modification) and mounted in paraffin. Serial sections were cut at 5.0 micra. The slides were stained with hematoxylin-eosin, aqueous toluidine blue, and Rinehart's modification of the colloidal iron stain (Hale).

The patients studied included 6 with primary thyroidal myxedema, 6 with myxedema secondary to hypopituitarism (4 with Sheehan's syndrome and 2 with chromophobe adenoma), and 7 controls without thyroid disease or other endocrine disorders.

RESULTS

The sections obtained from the patients in the control group did not contain significant deposits of extracellular mucoprotein. There was no evidence of any substance which stained metachromatically with toluidine blue or displayed an affinity for colloidal iron, except for minute traces seen occasionally near the fibroblasts around hair follicles and small blood vessels. The appearance of all specimens in this group was that of normal skin (Fig. 1).

It was not possible to differentiate histologically between primary and secondary myxedema. The changes in the connective tissue were, for all practical purposes, identical in skin obtained from untreated patients with one or the other condition. Deposits of extracellular metachromatic material and of colloidal iron were seen in the upper papillary layer of the dermis (corium) separating and disrupting the usual orderly, closely packed arrangement of the collagen fibers and bundles. The material was particularly prominent around vessels and hair follicles (Fig. 1).

In patients with primary or secondary myxedema who had received adequate replacement therapy, the histologic appearance of the skin was indistinguishable from that of the control groups. When treatment with desiccated thyroid was discontinued, there was a gradual, progressive return toward the picture seen in the severely hypothyroid state. The metachromasia was observed to appear first immediately below the basal layer of the epithelium, most profusely around blood vessels and hair follicles. The accumulation of the mucinous material was slow, taking three to five weeks to become clearly apparent. With the passage of longer periods of time, increasing amounts of the extracellular mucoprotein were visible, and more massive deposits were seen near the surface. There subsequently occurred greater disruption of the collagen bundles, and progressive extension of the process into the deeper layers of the dermis. In this later phase,

it was possible to recognize the myxedematous state from examination of the hematoxylin-eosin sections alone because of the separation of the collagen fibers and the haphazard arrangement of the bundles. The recurrence of the myxedematous state could be recognized histologically several weeks before it was apparent clinically.

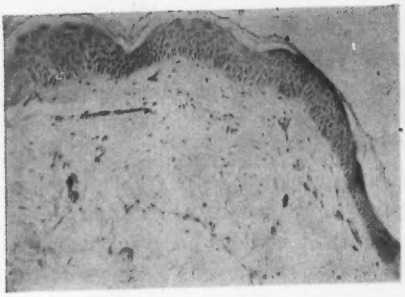
The disappearance of the metachromatic substances after treatment was begun or reinstituted took place somewhat more rapidly than the process of accumulation. Again, there was considerable variation between patients, depending primarily on the severity of the myxedematous changes at the time therapy was started. Some diminution was observed as early as one week after the onset of thyroid therapy, but two to three weeks of treatment were required for a significant reduction to be noted histochemically. In the patients with pituitary myxedema, administration of the thyrotropic fraction of the adenohypophysis (TSH) was as effective as desiccated thyroid in restoring the findings to the normal pattern, but it had no effect on the skin of the group with primary thyroidal myxedema.

The dosage of desiccated thyroid was also of importance in determining its effect on the skin. Complete disappearance of the mucinous deposits was never obtained with less than 120 mg. per day. Smaller amounts produced a significant diminution in the concentration of mucoprotein, but failed to restore the histologic appearance to normal, even after prolonged administration.

Attempts were made to evaluate the changes in the fibroblasts and capillaries in relation to evolution and retrogression of the myxedema. There seemed to be an increase in the size and basophilia of the fibroblasts, associated with both active accumulation and dissolution of the extracellular interfibrillar substances; in some sections capillary budding was observed during both of these phases. The observations were neither constant nor clear-cut, so that no conclusions could be drawn. No attempt was made to evaluate changes in the epithelium, since our interest was centered on the responses in connective tissue.

DISCUSSION

The results of this investigation indicate that in the absence of an adequate supply of thyroid hormone, whether due to primary thyroidal deficiency or to pituitary disease, an accumulation of metachromatic material is found in the connective tissue of the skin. These extracellular interfibrillar deposits are thought to consist of combinations of a protein or proteins with the mucopolysaccharides, hyaluronic acid and chondroitin sulfuric acid (4 and 6–9). The latter have both been isolated from human and animal skin. Chemical studies by others suggest that the difference between normal and myxedematous tissues represents a quantitative ra-



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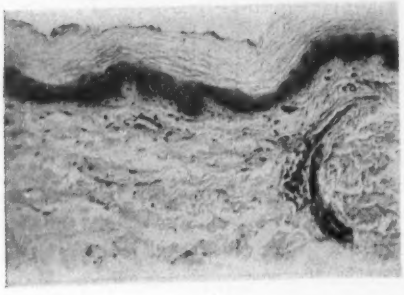

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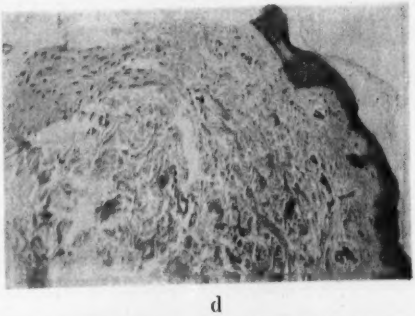

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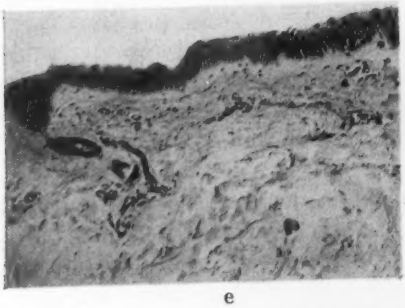

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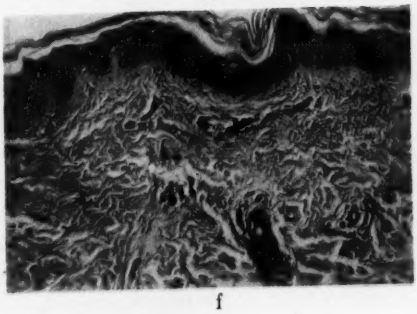

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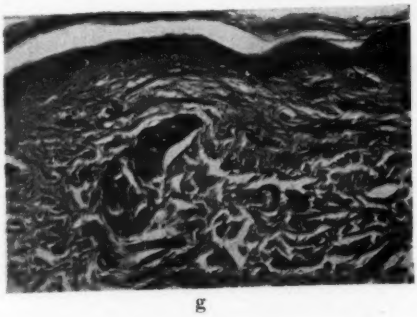

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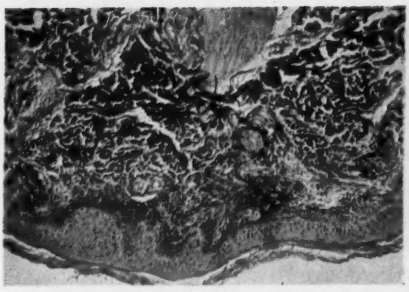
FIGURE 1, a-g
(Legend on facing page)



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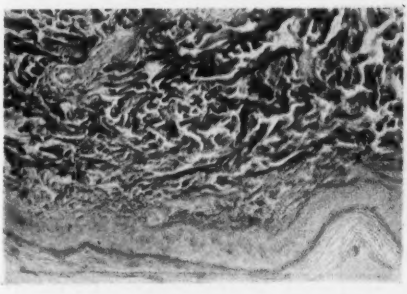

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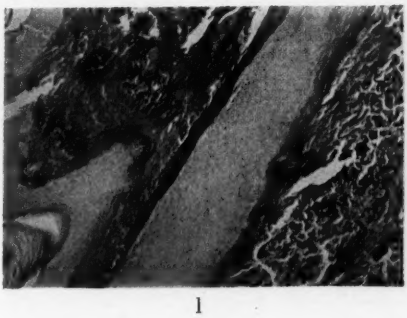

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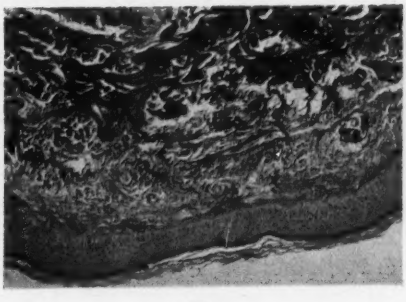

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FIGURE 1, h-m

1. Toluidine blue stain.
   a) Normal control.
      There is a negligible quantity of metachromatic material.
   b) Primary myxedema, untreated.
      Note the large amount of pink metachromatic material in the extracellular space.
   c) Primary myxedema, treated for five weeks with desiccated thyroid.
      Note the reduction in metachromasia.
   d) Primary myxedema, treated for three months with desiccated thyroid.
      Note the complete disappearance of metachromatic material.
   e) Primary myxedema; no treatment for three weeks.
      The metachromatic substance has reappeared.
   f) Pituitary myxedema untreated.
      The metachromatic material is present in appreciable quantities.
   g) Pituitary myxedema, treated with TSH for one week.
      Note the reduction in metachromasia.

2. Rinehart stain (modification of colloidal iron stain).
   h) Normal control.
   i) Primary myxedema, untreated.
      Note the large amount of blue-staining colloidal iron.
   j) Primary myxedema, treated for three months with desiccated thyroid.
      Note the disappearance of colloidal iron.
   k) Primary myxedema; no treatment for three weeks.
      Colloidal iron is again present.
   l) Pituitary myxedema, untreated.
      Colloidal iron is present in appreciable quantities.
   m) Pituitary myxedema, treated with TSH for one week.
      Note the reduction in colloidal iron.

ther than a qualitative change in the mucinous substances normally present in the skin (4, 10).

The nature of the staining reactions requires discussion. With aqueous toluidine blue, most cellular and extracellular elements are stained blue, whereas mucopolysaccharides (and probably some proteins) in a highly polymerized form are stained a definite red-violet. This reaction is not a specific one, and is probably more closely related to the degree of polymerization of the stained material than to its chemical nature. However, experience has shown that in the skin the concentration of metachromatic substances varies directly with the hexosamine content, and that the stained material can be removed by incubation of the tissue sections in hyaluronidase (11). The amount of metachromatically stained matter in a section has, therefore, been accepted as a guide to the concentration of polysaccharide in the extracellular space. The reaction is only roughly quantitative, but can provide valuable information. The colloidal iron stain is generally considered less specific and less discriminating. However, it is worth noting that the correlation between metachromatic staining and iron deposits in the sections studied was excellent.

The amount of material which accumulates in the dermis is dependent on the degree and duration of deficient thyroid function. Following omission of thyroid therapy in patients with marked thyroid deficiency, a period of three to five weeks had to elapse before the changes in the skin became apparent histologically. This interval, although long enough to permit the observation of metachromasia in skin biopsies, was not long enough to permit the appearance of clinically evident myxedema. The opposite effect—disappearance of the mucinous substances after treatment with desiccated thyroid—was also observed. The rate at which this occurred depended on the amounts of metachromatic material present at the outset. In the case of a previously untreated patient with large deposits, a significant diminution was apparent in two weeks; but a period of six to eight weeks was required for complete elimination of the excessive mucinous matter. However, if inadequate dosages of desiccated thyroid were employed, residual material remained visible indefinitely.

These observations were found to be valid in both primary myxedema and myxedema of pituitary origin. It has long been known that mucinous deposits occur in the connective tissues of the former group of patients, but the fact that identical changes take place in the skin of those suffering from a lack of pituitary thyrotropin has not been previously reported.

The mechanism by which the increased quantity of connective tissue polysaccharide is induced is worthy of some speculation. Since all previous investigations of this material have been based on studies of tissues from patients with primary or localized myxedema, the suggestion has been

made that the deposition of mucoprotein is the result of the action of elevated levels of thyrotropin (TSH) or an associated pituitary fraction directly upon the connective tissues, with a consequent increase in the local production of mucopolysaccharides (8). This concept is no longer tenable, in view of the finding in this investigation of identical changes in the skin of patients with pituitary deficiency in whom circulating levels of TSH must be extremely low or absent. Therefore, the factor which initiates the myxedematous process is most likely a deficiency of thyroid hormone (or its tissue-active form) at the periphery.

We have found in other studies (12) that the 24-hour urinary excretion of hexosamines is very low in both primary and pituitary myxedema. The amount excreted in twenty-four hours falls to approximately half that found in normal controls. This observation has been confirmed by Boas (13). In addition, we have found that administration of desiccated thyroid to these patients increases the hexosamine output significantly, although not to normal levels in the dosages used (12, 14). These findings suggest that the rate of breakdown (depolymerization) of the mucoprotein is influenced by thyroid activity. Thus the rate of breakdown of the mucinous substance to soluble and excretable molecular size may be materially decreased in the hypothyroid state, whereas the rate of formation may remain constant or be only slightly reduced. Such a situation would result in an increased accumulation of mucin in the connective tissues. It must be pointed out that this hypothesis assumes the presence of a depolymerizing enzyme such as hyaluronidase in the connective tissues, and that no such enzyme system has as yet been demonstrated.

SUMMARY AND CONCLUSIONS

Histochemical studies of the changes in the skin of patients with primary and secondary myxedema have been carried out by means of serial punch biopsies. The histologic findings were similar in both disorders. These consisted of the extracellular deposition of metachromatically stained material, interpreted as being a mucopolysaccharide-protein complex. During the administration of desiccated thyroid, there was a decrease in the amount of this material and a return to the normal histologic appearance of skin. Following withdrawal of replacement therapy, the histologic evolution of the myxedematous state was observed.

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EFFECT OF ADRENOCORTICOTROPIC THERAPY ON THE *IN VITRO* 11β-HYDROXYLATION OF DEOXYCORTICOSTERONE BY HUMAN ADRENAL HOMOGENATES

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THE hydroxylation and secretion of steroids by the adrenal cortex has been studied extensively in animals (1, 2), and there is a marked species difference in the quantitative and qualitative nature of the secretion. The perfusion of surgically removed human adrenals with media containing adrenocorticotropic hormone (ACTH) yielded only traces of cortisol (11β,17α,21-trihydroxy-4-pregnene-3,20-dione) and corticosterone (11β,21-dihydroxy-4-pregnene-3,20-dione) (3), yet these are the principal substances obtained on perfusion of ox adrenals (4) and are two of the major steroids found in human adrenal venous blood. Pincus and Romanoff (5) isolated crystalline cortisol, corticosterone and 11β-hydroxy-4-androstene-3,17-dione from human adrenal venous blood. Hudson and Lombardo (6) observed wide variations in the amount of cortisol and corticosterone in the adrenal venous blood of 3 women with breast cancer. When corticotropin (ACTH) was administered to 1 of these patients during the collection of blood the total steroid output in unit time was increased in association with an increased flow of blood through the gland rather than with an increase in steroid concentration in the adrenal vein blood. Balfour (7) observed the same effect of corticotropin in a calf. Sweat (8) reported a two- to fivefold increase in peripheral venous blood following administration of corticotropin. Little attention has been paid to the histologic appearance of the glands; reports have merely stated that the adrenals were "normal" or contained tumor cells. In view of the variations in chemical composition and cytology of the human adrenal cortex in stress and after corticotropin therapy (9, 10), it is important to correlate changes produced in adrenal vein steroids as a result of corticotropin administration with corresponding morphologic changes in the adrenal cortex (11). Similarly the marked difference in hydroxylation between bovine and human glands can be explained when these quantitative enzyme studies are correlated with histologic changes in the gland. The investigations now reported are

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concerned principally with the effect of corticotropin administered *in vivo* on the 11β-hydroxylation of steroids by human adrenal glands *in vitro* and to a lesser extent with its effect on the adrenal vein steroids.

METHODS

Adrenal glands were obtained from 13 patients with metastatic mammary carcinoma undergoing two-stage bilateral adrenalectomy, and were placed in beakers standing in crushed ice. At the time the first gland was removed, 11 of the patients had had neither corticotropin nor steroid hormone treatment. The second operation took place after an interval of about three weeks. For three days prior to, and on the morning of the day of the second operation, 9 of the patients received 100 units of corticotropin (ACTHAR gel) daily. Corticotropin was administered to 2 patients (Cases 3 and 5, Fig. 1A) before the first operation instead of the second. In 2 other patients (Cases 10 and 11, Fig. 1A) no corticotropin was given at any time. In addition, a few postmortem glands were examined within four hours after death, and ox adrenal glands were obtained in a fresh state.

Homogenates. Blood, fat and connective tissue adhering to the glands were removed, and the adrenals cut into slices a few millimeters thick. One or more slices, including those made through obvious abnormalities such as metastatic tumors, were fixed in 10 per cent formol-calcium solution for histologic examination. The remaining slices were used for assay of 11β-hydroxylation after removal of blood clot within the gland

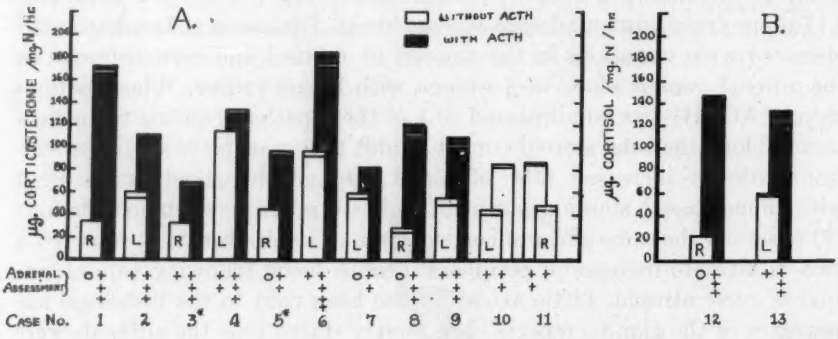

FIG. 1. *Effect of corticotropin (ACTH) administration on formation of corticosterone* (**A**) *and of cortisol* (**B**) *in vitro.* Steroid 11β-hydroxylation of homogenates of adrenals obtained at two-stage bilateral adrenalectomy from a series of patients with carcinoma of the breast. In 9 cases, ACTH was administered before the second operation. In Cases 3 and 5 (asterisks) the ACTH was given before the first operation instead of the second. In Cases 10 and 11 no ACTH was given at any time.

Incubation took place in air for one hour at 37° C. Reaction mixture contained 95 mM potassium chloride, 4 mM magnesium sulphate, 40 mM potassium phosphate (pH 7.4), 10 mM potassium fumarate, 0.4 mM-ATP, 0.2 mM-TPN$^+$, about 200 mg. wet weight adrenal tissue and 0.04 ml. propylene glycol containing: (**A**) about 500 μg. DOC, or (**B**) 17-hydroxy DOC; total volume 3 ml. Double lines at the ends of bars indicate duplicate values. The histologic assessment of the glands (shown below) is based principally upon the degree of lipid depletion. L indicates left, and R right gland.

and any obvious tumor tissue. The following operations were performed at 0° C. The slices were ground in a small Latapie mincer and the pulp obtained was weighed (about 2 Gm.) and homogenized with approximately 3 volumes of 0.25 M sucrose solution containing 0.12 M nicotinamide for two minutes in a glass tube with a nylon pestle (12). Care was taken to maintain a standard procedure in preparing the homogenates, and the absence of unbroken cells was checked by microscopic examination of stained smears. Homogenates were finally diluted with sucrose-nicotinamide solution to contain 200 mg. of wet-weight adrenal pulp per ml. The total nitrogen content of a portion of the homogenate, dialyzed free from sucrose and nicotinamide, was determined by Nesslerization after digestion with sulphuric acid and mercury catalyst.

Incubation, and determination of steroids in adrenal homogenates. Deoxycorticosterone (DOC) (21-hydroxy-4-pregnene-3,20-dione) and 17α-hydroxy-DOC (17α,21-dihydroxy-4-pregnene-3,20-dione) were incubated with adrenal homogenates under the conditions and with the controls described by Brownie and Grant (12). DOC and corticosterone were extracted from the incubated mixtures and determined by the methods of Brownie and Grant (12) modified by Grant (13). The method employed for corticosterone was also used for 17α-hydroxy DOC. This was possible, since the two steroids did not occur together in the same mixtures. Cortisol extracted by chloroform was separated by paper chromatography and determined by the method described subsequently for the analysis of adrenal venous blood.

Histologic methods. Sections were prepared and stained for morphologic and histochemical changes as described previously (11). The adrenal patterns were assessed 0 to ++++ depending mainly on the degree of lipid depletion (Table 1) and to a lesser

TABLE 1. ASSESSMENT OF ADRENAL PATTERNS

0	= Normal lipid-laden adrenal.
+	= Focal lipid depletion involving 1–25 per cent of cortex.
++	= Focal lipid depletion involving 25–50 per cent of cortex.
+++	= Focal lipid depletion involving 50–95 per cent of cortex.
++++	= Almost complete or extreme diffuse lipid depletion.

extent on cytology (14). This assessment was made in the Pathology Laboratory by workers who were not aware of the results of the hydroxylation experiments.

Extraction, and determination of steroids in adrenal venous blood. Blood was collected at operation from the adrenal vein by means of a polythene cannula and received in a container cooled in ice and water. Red cells were centrifuged off without delay. Cortisol and corticosterone were determined in the plasma by the method described by Vogt (15) modified as follows. It was found most satisfactory to elute steroids from the paper by vigorous hand shaking in 30-ml. glass-stoppered tubes. The colored solution finally obtained was diluted to 3.0 ml. and the optical density was measured at 510 mμ in a Unicam S.P. 500 spectrophotometer. With these modifications, over 90 per cent of added cortisol (10 μg.) and corticosterone (10 μg.) could be recovered from 5 to 10 ml. of human peripheral blood plasma.

RESULTS

11β-Hydroxylation in human adrenals

Preliminary experiments in which 500-μg. portions of DOC or 17α-hydroxy-DOC were incubated with 200 mg. (wet weight) of homogenized

human adrenal tissue indicated that it was necessary to add 10 mM of fumarate, 0.4 mM of ATP and 0.2 mM of TPN^+ in order to obtain reasonable metabolism of the added steroids.

No metabolism of DOC or 17α-hydroxy-DOC was observed with human adrenal glands obtained post mortem, even when they were removed within two hours of death. Glands removed surgically remained active for at least eighteen hours if cooled at once to 0° C. and stored at this temperature. In the experiments reported the glands were used without delay.

Paper chromatography (by the methods of Bush (16)) of steroids extracted from incubation mixtures with chloroform suggested that corticosterone was the principal product derived from DOC, and cortisol from 17α-hydroxy-DOC. Substances running to the same positions as authentic corticosterone and cortisol on the paper chromatogram produced the blue-tetrazolium reaction of Mader and Buck (17), and on elution from the paper their absorption spectra in ethanol and sulphuric acid (18) were identical with the spectra of authentic corticosterone and cortisol. Thus although absolute identity has not been established for the products obtained on incubation of DOC and 17α-hydroxy-DOC with human adrenal homogenates, it would appear very probable they are corticosterone and cortisol respectively.

The 11β-hydroxylating activity of homogenates from a series of human adrenal glands when DOC and 17α-hydroxy-DOC were used as the substrate are shown in Figure 1, A and B. It will be observed that when a dose of 300–400 units of corticotropin (ACTH) was given to the patient up to the time of operation, the glands exhibited a marked increase in 11β-hydroxylating activity. This increase was found when corticotropin was given before either the first (Cases 3 and 5) or the second operation. In such glands there was a distinct alteration in morphology, the zona reticularis and zona fasciculata appearing as one zone (Fig. 2), and there were significant changes in the cells of zona fasciculata. Their lipid content diminished; the globules become fine, and dispersed throughout the cytoplasm of the cell. The lipid-depleted cells (haemalum and eosin preparations) appeared compact and special stains showed that they were rich in ribonucleic acid (RNA), acid and alkaline phosphatase and the different enzymes of the tricarboxylic acid cycle (11). Such glands are designated as +++ or ++++ lipid depletion. In the same patient, when the contralateral gland was removed without any previous hormone therapy, the 11β-hydroxylating activity was very much lower. This lower hydroxylation was likewise found in the second adrenals of the patients (Cases 3 and 5) who had received corticotropin before the first operation but no supportive hormone therapy prior to the second operation. Such glands appeared nor-

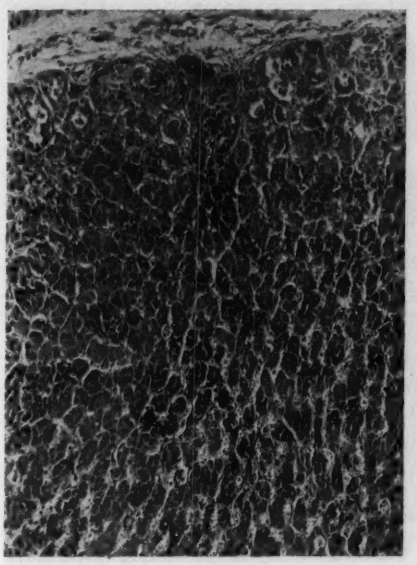

FIG. 2. *Human adrenal after stimulation with corticotropin (ACTH).* The whole cortex consists of columns of compact cells. The zona reticularis and zona fasciculata consist of a single zone. (H. & E. ×50)

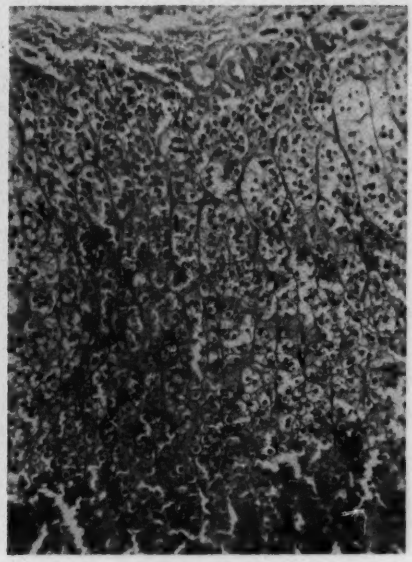

FIG. 3. *"Normal" human adrenal—no hormone therapy.* The narrow zone reticularis, seen below, is sharply defined from the overlying zona fasciculata, the cells of which appear vacuolated (clear cells). (H. & E. ×50)

mal histologically (Fig. 3). The zona fasciculata and zona reticularis were sharply demarcated, and the cells of the zona fasciculata (clear cells) were full of large lipid globules, and very poor in RNA, alkaline and acid phosphatase and the Krebs cycle enzymes. The cells of the zona reticularis were smaller, but compact in type and rich in RNA and enzymes.

It is notable that in certain patients (Nos. 4 and 11), who received no corticotropin, lipid depletion was marked (++) and there was distinct broadening of the zona reticularis (Fig. 4). Marked increase in 11β-hydroxylating activity was present. This is presumably due to the patients' endogenous corticotropin as a result of preoperative stress.

Similar results were obtained in a smaller series of experiments when 17α-hydroxy DOC was used as substrate (Fig. 1B).

11β-Hydroxylation in ox adrenals

For the sake of comparison, several experiments were performed with ox-adrenal homogenates under the conditions used with human glands. The ox adrenals were much more active than the human glands. When 500 μg. of

FIG. 4. *Human adrenal—no hormone therapy.* Note the increase in width of the zona reticularis. This is associated with an increase in 11β-hydroxylation. (Case 4, left adrenal, Fig. 1A.) (H. & E. $\times$50.)

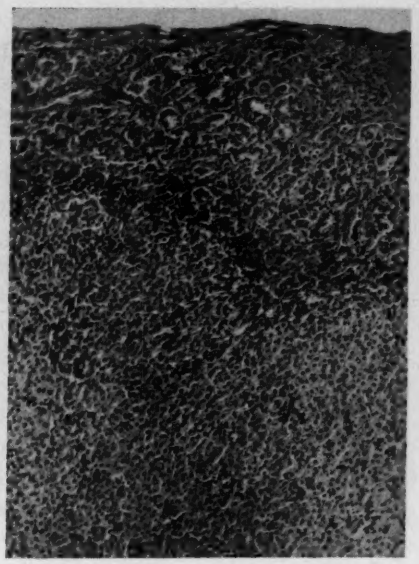

FIG. 5. *Ox adrenal.* A prominent zona glomerulosa is present on the surface of the gland. It is sharply defined from the underlying cortex which has a uniform appearance and is not differentiated into zona fasciculata and zona reticularis. H. & E. $\times$50.)

DOC was incubated with only 100 mg. (wet weight) of homogenized adrenal tissue the corticosterone formation invariably exceeded 300 μg./mg.N/hr. Histologically, the ox adrenal consists of a prominent zona glomerulosa easily separated from the rest of the adrenal cortex which consists of a uniform layer of cells (Fig. 5). There is no differentiation into fasciculata and reticularis as in the human gland. Although the zona glomerulosa is rich in lipid, the rest of the gland is poor in this material, and the little present appears as fine globules. The overall appearance of the ox adrenal resembles that previously described for human glands removed surgically after corticotropin therapy or after severe stress such as extensive burning. However, the compact cell (Fig. 6) is never so prominent as it is in the human gland (Fig. 7); it is more vacuolated and less eosinophilic but still rich in RNA. Although administration of corticotropin produced a marked change in the histologic appearance of the human adrenal cortex, no obvious histologic change was seen in the bovine (calf) gland when the animal received 600 mg. of corticotropin over six

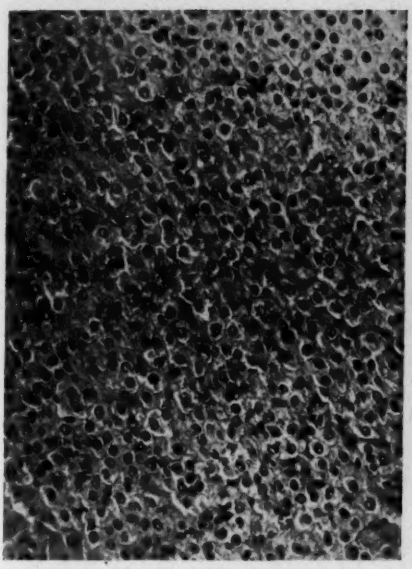

FIG. 6. *Ox adrenal.* Showing the solid appearance of cells in the cortex. There is not the same alveolar pattern as seen in the human cortex. (H. & E. ×215.)

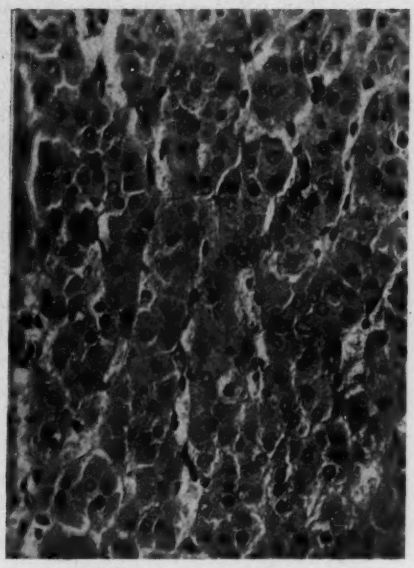

FIG. 7. *Human adrenal.* Showing arrangement of the compact cells seen in Figure 2. Compare with Figure 6. (H. & E. ×215.)

days, or when the animal was stressed. Again, the lipid-laden clear cell observed in the fasciculata of glands removed surgically from human subjects untreated by corticotropin or after death without severe stress, did not occur in the ox adrenals, even when the animals were rested for at least twenty-four hours and killed after stunning with the trap-bolt pistol.

Adrenal vein steroids

The adrenal vein was canalized at operation in cases in which a large single vein was found and it was possible to collect the complete venous drainage of the gland uncontaminated by blood from other sources. The arterial supply and the gland itself were undisturbed before and during the blood collection. It was possible in only 1 patient to carry out the canalization on both sides. Amounts of free cortisol and corticosterone found in the blood plasma, and the histologic assessments of the glands are shown in Table 2. Values found by Hudson and Lombardo (6) are also listed for comparison. It will be observed that, in agreement with these workers, the output of cortisol in unit time was increased in association with an increased flow of blood from the gland under the influence of corticotropin. The output of corticosterone was not increased.

TABLE 2. ADRENOCORTICOSTEROIDS IN HUMAN ADRENAL VENOUS BLOOD

Blood flow (ml./min.)	Cortisol		Corticosterone		Histologic assessment
	μg./ml.	μg./min.	μg./ml.	μg./min.	
Present investigation					
		After ACTH			
1.8	3.7	5.8	0.33	0.59	+++
2.0	3.8	7.6	0.66	1.32	+++
6.0*	5.3	32.0	1.45	8.0	++++
		Without ACTH			
1.25	2.8	3.5	2.3	2.87	+
1.9*	6.95	13.3	3.74	7.1	+
Hudson & Lombardo (6)					
		After ACTH			
2.1	6.8	14.3	2.3	5.8	None
		Without ACTH			
1.1	10.1	11.1	2.4	2.6	None

* Same patient.

DISCUSSION

When considering the significance of these results, emphasis must be laid on the fact that the adrenals were obtained from subjects with disseminated carcinoma of the breast, and accordingly may not be normal. However, the histologic appearance and histochemical response were those of a normal gland, and no evidence has been reported of significantly abnormal adrenocortical steroid biosynthesis or metabolism associated with breast cancer. Engstrom, Engbring and Schroeder (19) have reported the development of breast carcinoma in a young woman three and a half years after adrenalectomy for Cushing's syndrome. The fact that she had been maintained on small constant doses of cortisone and deoxycorticosterone suggested that the part, if any, played by those substances was a "permissive one."

The advantages have been stressed (11) of studying the effects of corticotropin on human adrenal glands obtained in the manner described—at bilateral adrenalectomy in two stages, the control being provided by the gland removed when the patient was untreated with exogenous hormone. This technique has proved valuable in the present series, since the human adrenal is of adequate size for convenient enzyme studies. Furthermore, there is an increased output of adrenocortical hormone (cortisol) in response to corticotropin stimulation, and although the concentration of hormone in the adrenal effluent may fall slightly, the amount secreted per

minute rises threefold. This is consistent with the two- to fivefold increase in steroid found in the peripheral blood by Sweat (8).

Correlation of the histologic picture with an increased production of adrenocortical hormone following administration of corticotropin establishes beyond doubt that the morphologic and histochemical changes observed in the adrenocortical cells can be interpreted in terms of activity. Thus the "compact cell," which is lipid depleted and rich in RNA and enzymes, can be considered in this instance as an active cell. This is a matter of some importance, since it is not justifiable to assume a similar state of activity in postmortem glands even though they are lipid depleted and rich in enzyme systems. Likewise, there is no justification for expressing the opposite view that the lipid-depleted adrenal cortex represents exhaustion, unless such a picture is associated with a failure to produce adrenocortical hormone (15).

The present investigation shows that increased activity of corticotropin-treated adrenal glands is associated with an increase in 11β-hydroxylation. This enzymatic activity results from the action of exogenous corticotropin and is not due to the effects of surgical stress, as shown by the low hydroxylation and almost normal histologic appearance observed in the second gland in Cases 3 and 5 (Fig. 1A). In addition, it has been established elsewhere (11) and confirmed here that morphologically the main response to exogenous corticotropin is seen in the cells of the zona fasciculata. The clear lipid laden cells found normally in this zone become depleted of lipid and show a quantitative increase in RNA and enzyme systems. This change from clear to compact cell is associated with a significant increase in 11β-hydroxylation, and is presumptive evidence that this enzyme reaction is taking place in or is associated with the occurrence of the compact cell. Final proof of this is possible only when the zones of the cortex can be separated in such a way as to obtain homogenates of clear and of compact cells. This aspect of the problem is at present under investigation and it is a matter of considerable importance to ascertain whether the low *in vitro* hydroxylation which occurs in normal or untreated glands is due to the small number of compact cells which constitute the zona reticularis. Likewise, the higher results (Fig. 1A, Case 4) seen occasionally in untreated glands and ascribed to the action of endogenous corticotropin, may be explained by the increase in width of what appears to be the zona reticularis (Fig. 4).

The results of this study confirm previous observations (11) that the main morphologic effect of exogenous corticotropin is seen on the cells of the zona fasciculata nearest the zona reticularis, and the zone of compact cells so produced gives the appearance of widening of the reticularis.

Although it is shown (Fig. 1A and B) that the administration of cortico-

tropin results in a marked increase of 11β-hydroxylation, on occasion the amount of hydroxylation is slight. In some instances low hydroxylation was associated with difficulty and delay in removing the gland, and in 1 case the second operation was postponed and the last injection of corticotropin given twenty-four hours before removal of the adrenal. The results of 11β-hydroxylation assays and the histologic assessments in this patient are shown in Table 3. This case illustrates once more the limitation of histologic

TABLE 3

	11β-Hydroxylation (μg. DOC/mg. N)	Histologic assessment
First adrenal removed (no ACTH)	33	0
Second adrenal removed (operation delayed 24 hours after last injection of corticotropin)	24	+++

methods by themselves, and the care that must be exercised in assessing functional activity from a static histologic picture. This applies particularly to postmortem glands which are susceptible to autolytic changes. The enzymes concerned with steroid 11β-hydroxylation appear to lose their activity rapidly under these conditions.

Comparison of hydroxylating activity of human and bovine adrenal cortex

The high activity of ox adrenals in effecting steroid 11β-hydroxylation is well known (12, 20, 21). This is not surprising in view of the histologic appearance of the adrenal cortex of the ox (Figs. 5 and 6) and its similarity to the cortex of the human gland subjected to stress or exogenous corticotropin stimulation (Fig. 2). The striking difference in histologic appearance between the ox adrenal cortex and normal human adrenal cortex (Fig. 3) may explain the different results found when ox and human glands are perfused with media containing corticotropin (4). It remains to be seen whether, instead of using the normal human lipid-laden gland, a greater yield of cortisol and corticosterone would be obtained from perfusion experiments using adrenals from patients who had received corticotropin. It is only possible, under such conditions, to compare the results of perfusion experiments between human adrenals and ox adrenals.

SUMMARY

1. Adrenal glands were obtained from 13 patients who were undergoing two-stage bilateral adrenalectomy for metastatic mammary carcinoma.

2. Using the first gland as a control, the effect of corticotropin administered *in vivo* was studied on the *in vitro* 11β-hydroxylation of deoxycorticosterone by human adrenal homogenates.

3. The action of corticotropin on the morphology and histochemistry of the cells of the zona reticularis and zona fasciculata has also been described.

4. In certain cases the adrenal vein was canalized and the levels of free cortisol and corticosterone estimated in the blood plasma.

5. The histologic picture following administration of corticotropin has been correlated with an increased production of adrenocortical hormone and increased 11β-hydroxylating activity, and it has been established that the morphologic and histochemical changes observed in the cells of the adrenal cortex can be interpreted in terms of function.

6. A comparison has been made of the hydroxylating activity of the human and bovine adrenal cortex; it is suggested that the human adrenal cortex on which corticotropin has acted *in vivo* may produce greater yields of cortisol and corticosterone in perfusion experiments with corticotropin.

Acknowledgments

We are indebted to the surgeons and physicians at the Royal and Western Infirmaries, Glasgow, and at the Western General Hospital, Edinburgh, for their excellent cooperation in this investigation. We also wish to acknowledge our appreciation to Miss A. K. Lunn and Mr. D. W. Davidson for assistance with the experimental work, and to Miss W. Smith for the histologic preparations.

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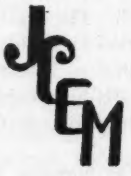

ADRENOCORTICAL CARCINOMA WITH GYNECOMASTIA: A CASE REPORT AND REVIEW OF THE LITERATURE

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THE development of gynecomastia is an uncommon result of excessive steroid hormone production by adrenocortical tumors. The present report concerns the thirty-fourth occurrence of this phenomenon recorded in the world literature (1–16). The findings of relative adrenocortical insufficiency, increased excretion of estrogen-like materials in the urine and the successful isolation of steroids from the tumor (17) are of special interest in this patient.

CASE REPORT

A 28-year-old radio announcer noted the onset of intermittently tender nipples in March 1953 and of bilateral breast enlargement in September 1953. There was progressive increase in size during the next twelve months. The patient was sufficiently embarrassed by this cosmetic defect to request admission to the Salt Lake Veterans Administration Hospital in March 1955 for evaluation and treatment.

He had noted a very gradual decline in libido and potency during the two years prior to admission. There were no other symptoms and no fluctuations in weight. The past history was negative. There was no history of gynecomastia during puberty, or of jaundice, liver disease, alcoholism or exposure to hepatoxins or estrogens.

The patient had a 2-year-old child. Contraception had been employed for two years prior to hospitalization.

Physical examination revealed a young, thin white male who appeared in good health. His voice was deep and masculine. The blood pressure varied from 120/60 to 160/100, the pulse was 84 per minute, respirations 15 per minute and temperature 98.6° F. The skin was clear. Pubic and axillary hair were present in moderate amounts and the pubic hair had a feminine distribution. The beard was sparse.

Examination of the head and neck, lymphatic system, heart, lungs, extremities, rectum and neurologic system yielded negative results. The abdomen was free of palpable masses or enlarged organs. The breasts were considerably enlarged (Fig. 1), with palpable subareolar glandular tissue, deeply pigmented, non-tender nipples and prominent Montgomery tubercles. The testes measured 1.5 to 2 cm. in diameter and were smooth and firm. The penis and prostate were normal.

Laboratory data. The volume of packed red blood cells was 41 ml. per 100 ml. of

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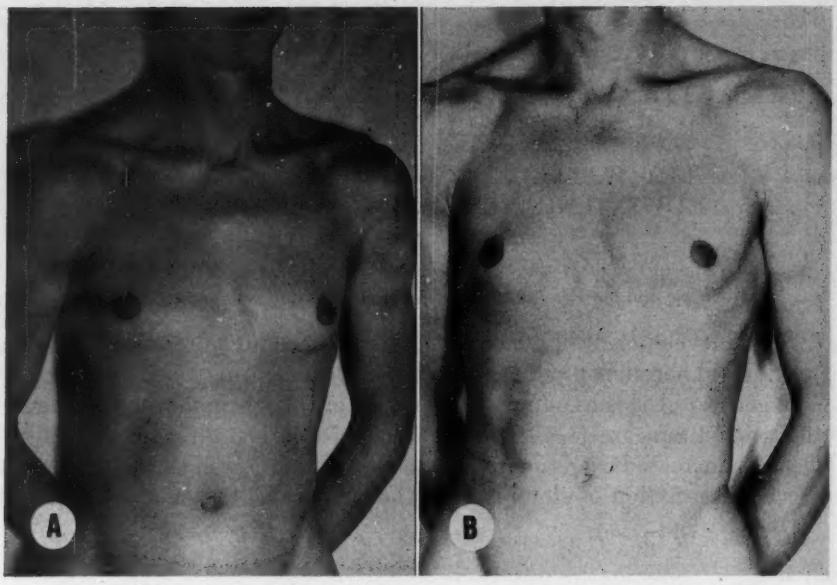

FIG. 1. Breasts of Patient W.L.: **A.** Before removal of adrenocortical carcinoma. **B.** Twelve months after the operation.

blood. The leukocyte count was 6,650 per cu.mm. with 67 per cent neutrophils, 31 per cent lymphocytes, 1 per cent monocytes and 1 per cent eosinophils. A peripheral blood smear revealed normochromic, normocytic erythrocytes and adequate platelets. Results of urinalysis were negative. The blood urea nitrogen level was 5.7 mg. per 100 ml. The concentration of serum sodium was 151 mEq./L., chloride 109 mEq./L., potassium 3.4 mEq./L. and bicarbonate 27 mEq./L. The concentration of serum total protein was 5.9 grams per 100 ml., of which 4.4 grams was albumin. The total serum bilirubin level was 0.6 mg. per 100 ml., with 0.1 mg. of direct-reacting bilirubin per 100 ml. The serum thymol turbidity was 3.5 units and the serum alkaline phosphatase level 1.8 Bodansky units per 100 ml. The concentration of serum cholesterol was 164 mg. per 100 ml. Serum bromsulfophthalein retention at forty-five minutes was 1 per cent. The serum protein-bound iodine level was 4.1 μg. per 100 ml.

An oral glucose tolerance test showed a fasting blood sugar concentration of 79 mg. per 100 ml. with values of 95, 82, 71 and 100 mg. per 100 ml. at a half hour, one hour, two hours, and three hours, respectively, after ingestion of 50 grams of glucose. During an intravenous glucose tolerance test utilizing a thirty-minute infusion of 0.5 Gm. of glucose per Kg. of body weight, the maximum blood sugar concentration was 184 mg. per 100 ml. at the end of the infusion, with a return to a level of 80 mg. per 100 ml. one hour later. No hypoglycemia followed either test.

Seminal fluid obtained by ejaculation was turbid and watery. The sperm count was 1.7 million per ml. Forty per cent of the sperm were motile and less than 5 per cent were atypical.

Roentgenograms showed a normal chest, skull, pelvis and long bones. Upon abdom-

FIG. 2. Combined intravenous pyelogram and presacral air insufflation, showing large ovoid mass above the right kidney, displacing the kidney downward and the liver anteriorly.

inal x-ray examination, there was a suggestion of a mass in the right upper quadrant. A combined intravenous pyelogram and presacral air insufflation study demonstrated a large ovoid mass above the right kidney, displacing the kidney inferiorly and the liver anteriorly (Fig. 2).

The Friedman test for chorionic gonadotropin in the urine yielded negative results. Upon bioassay for urinary follicle-stimulating hormone (FSH) performed by the method of Salhanick (18), there was no detectable FSH. In human adult males, the normal range is 2 to 3 Armour units.

Urinary 17-ketosteroid excretion (19) varied from 8.6 to 13.2 mg. per twenty-four hours (normal range, 10 to 15 mg.). Urinary 17-hydroxycorticosteroid excretion (20) varied from 4.5 to 7.1 mg. per twenty-four hours (normal range in this laboratory, 1.0 to 7.7 mg.).

The plasma free 17-hydroxycorticosteroid (17-OHCS) level (21) at eight o'clock in the morning varied from 15 to 18 μg. per 100 ml. Serial measurements of plasma free 17-OHCS during a 24-hour period revealed a progressive decline from 16 μg. per 100 ml. in the early morning to a minimum value of 8 μg. per 100 ml. at midnight. A rapid rise in the concentration of plasma free 17-OHCS occurred after four o'clock in the morning. The mean level of plasma free 17-OHCS for normal persons at eight o'clock in the morning in our laboratory is 12.5 ± 3.7 μg. per 100 ml., and a diurnal variation similar to that described for the patient is seen in normal individuals (22).

Tests of response to corticotropin (ACTH) and cortisol removal were performed in

accordance with previously described protocols (23, 24). Figure 3 depicts the patient's responses to six-hour infusions of 25 units of ACTH administered at various times during an eighteen-month period. Prior to surgery, there was no rise in the level of plasma free 17-OHCS, in contrast to an approximate threefold rise in normal persons. The cortisol removal test revealed a moderate delay in the disappearance of plasma free 17-OHCS after a standard thirty-minute infusion of 1 mg. of cortisol per Kg of body weight.

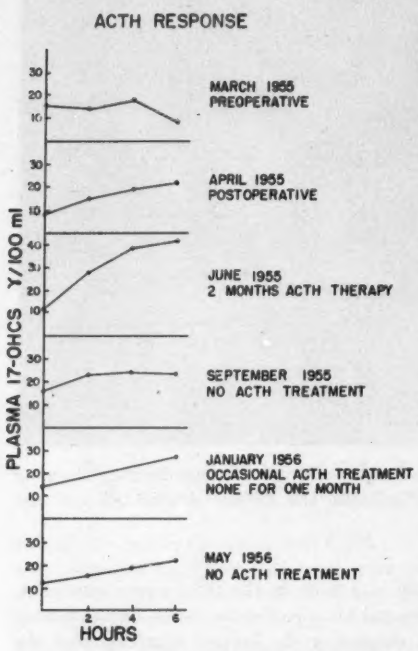

FIG. 3. Plasma 17-OHCS levels after standard ACTH-stimulation tests.

Bioassay for urinary estrogens was performed by Dr. R. K. Meyer of the University of Wisconsin, utilizing a modification of the vaginal cornification technique of Allen and Doisy (25). The urinary estrogen content varied from 73 μg. to 177 μg. of estrone equivalent per twenty-four hours. The normal range for adult males is from 2 to 29 μg.

Operation. On the fourteenth hospital day, Dr. J. A. Gubler explored the patient's abdomen through a right flank incision. Because of poor augmentation of adrenocortical secretion of 17-OHCS by exogenous ACTH (Fig. 3), 150 mg. of cortisone was given on each of the two days prior to operation, and 100 mg. of cortisol was administered intravenously during surgery. A large solid tumor occupied the right suprarenal area. No evidence of local invasion or intra-abdominal metastases was found and the opposite adrenal was normal to palpation. Excision was performed without incident.

The tumor was a firm encapsulated oval mass, weighing 920 grams (Fig. 4). Numerous large veins traversed the thick fibrous capsule. A cut section showed a lobular contour with bulging of the tumor tissue above the cut surface. The tumor was predominantly grey-brown in color with some dark brown and yellow areas. There were small foci of necrosis and two small cysts.

On microscopic examination, the tumor consisted of sheets, nests and cords of pleomorphic epithelial cells. Many cells were small and regular with oval, pale, blue-staining nuclei. Others were large and polyhedral with abundant cytoplasm and hyperchromic, often multilobed nuclei. Multinucleate cells were not uncommon. In certain areas, invasion of the fibrous capsule and of the walls of capsular veins was seen (Fig. 5).

Explantation of tumor cells into the anterior chamber of the eyes of 4 guinea pigs resulted in gross proliferation of the tumor. Pathologic diagnosis by Dr. O. N. Rambo was cortical carcinoma of the right adrenal gland with evidence of invasion of capsular veins.

The steroid content of the tumor was determined by Drs. Salhanick and Berliner (17). They identified two compounds, progesterone and equilenin, in concentrations of 850 and 580 μg. per 920 Gm. of tumor, respectively. Six other steroids were isolated in

FIG. 4. Gross appearance of transverse section of adrenocortical carcinoma.

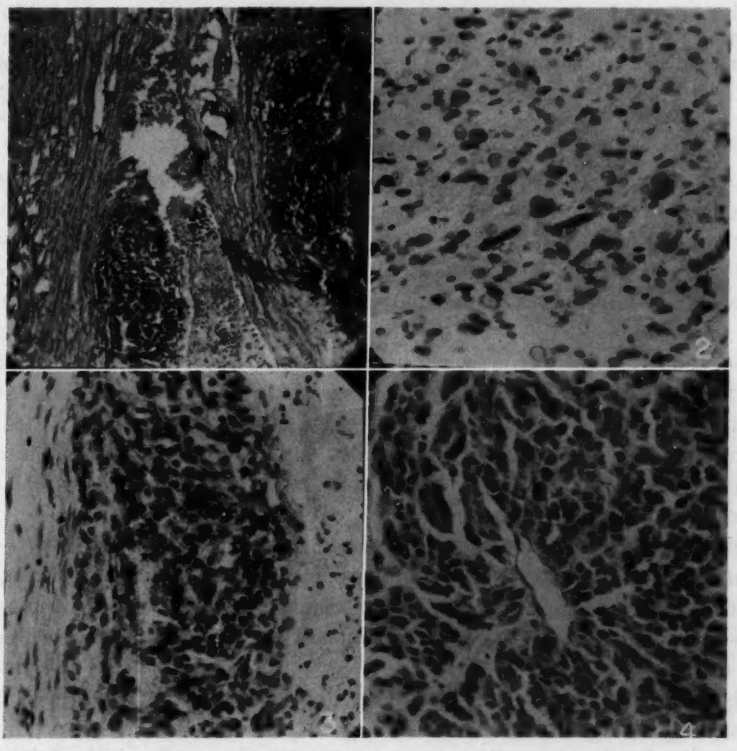

FIG. 5. Photomicrographs of various portions of the adrenocortical carcinoma. Note the metastatic cells in the vein in section 1; the same area under higher magnification in section 3; the pleomorphism of the malignant cells in section 2; and the relation of the malignant cells to a blood vessel in section 4.

lesser amounts, but precise identification of these latter six compounds was not possible. Some were shown to be phenolic, thus having benzenoid A rings. These were not estradiol, estriol or estrone. Other compounds among the unidentified group were 17-ketosteroids. These findings are to be reported in detail elsewhere (17).

The postoperative course was uneventful. Moderate doses of cortisone and, later, cortisol were given during the first and second postoperative weeks. The dosage of cortisol was gradually reduced and finally discontinued in the third postoperative week. ACTH-gel was administered during the last four days of steroid therapy.

The patient resumed full activity four weeks after operation. Physical and roentgen examinations eighteen months after surgery revealed no evidence of recurrence or metastases. The gynecomastia had regressed completely. The breasts were slightly prominent but no glandular tissue was palpable (Fig. 1). The testes had doubled in size and the sperm count had gradually increased to 26 million per ml., with normal motility and structure. The physical findings were unchanged two years after surgery despite the appearance of pulmonary metastases.

A postoperative corticotropin test revealed continued poor adrenocortical response to ACTH (Fig. 3). Intensive therapy with corticotropin for the next two months resulted in a return to a normal response with this test. However, when corticotropin was discontinued for three months, a reversion to a suboptimal response occurred. Fourteen months and eighteen months after surgery, a twofold increment occurred in the plasma free 17-OHCS level after six hours of stimulation with corticotropin.

DISCUSSION

A summary of the clinical and biochemical features of the 34 reported cases of feminizing adrenocortical carcinoma is presented in Tables 1 and 2. Four cases prior to 1948 (2, 3, 4, 16) were not included in Wilkins' review of 12 patients (1). Cahill's patient (2) had a typical history, and a markedly enlarged left adrenal shadow was demonstrated by perirenal air insufflation. Hurxthal and Musulin (3) thought their patient had "a tumor of mixed renal origin." However, the pathologic findings, clinical features and the positive results of estrogen bioassay are more compatible with a feminizing adrenocortical carcinoma. Östergaard's patient (4) had a tumor in the scrotum which was anatomically separate from the homolateral, atrophic testis. It resembled an adrenocortical carcinoma rather than a testicular tumor.

Seventeen acceptable cases have been reported since 1948 (5–16). Chambers' patient (26) is not included because he more likely had a metastatic choriocarcinoma of the testis. In his case, serial sections of the testes were not made in order to rule out a microscopic primary choriocarcinoma (see reference 27).

The symptoms and physical findings in the reported patients have been remarkably uniform. Gynecomastia often preceded other symptoms by many months. Occasionally, symptoms due to the mechanical effects of a large tumor or the presence of reduced libido and potency were the chief complaints. In these patients, gynecomastia was usually present on ex-

TABLE 1. SUMMARY OF CLINICAL FEATURES OF 34 REPORTED CASES OF FEMINIZING ADRENOCORTICAL CARCINOMA

Case report and reference	Age of patient (yrs.)	Clinical findings				Remarks
		Palpable tumor	Gynecomastia	Testicular atrophy	Reduced libido & potency	
1. Bittorf, Mathias (1)	26	+	+	+	+	No treatment. Died of metastases 11 mos. after onset of symptoms
2. Zum Busch, Parkes-Weber (1)	27	0	+			No treatment. Died of metastases 3 mos. after onset of symptoms
3. Holl (1)	15	+	+	0		Tumor inoperable at exploration 2 mos. after onset of symptoms. Died a few wks. after surgery
4. Holl (1)	44	0	+	+	+	Tumor excised 2 yrs. after onset of symptoms. Regression of symptoms & no recurrence 6 mos. after surgery
5. Lisser (1)	33	+	+			No treatment. Died of metastases 2 mos. after onset of symptoms
6. Simpson & Joll (1)	34	+	+	+	+	Tumor excised 2 yrs. after onset of symptoms. Post-op. radiation. Recurrence & death from metastases 2 yrs. after surgery
7. Pico Estrada (1)	30		+	+		No treatment. Died of metastases
8. Pico Estrada (1)	41		+			No treatment. Died of metastases
9. Kooyman (16)	29	+	+	+	+	Tumor inoperable at exploration 3 yrs. after onset of symptoms. Died on first postop. day
10. Cahill *et al.* (2)	53	0	+	+	+	Symptoms present 3 yrs. Patient refused therapy or follow-up
11. Roholm & Teilum (1)	44	+	+	+	+	No treatment. Died of metastases 4 yrs. after onset of symptoms
12. Hurxthal & Musulin (3)	38	+	+	+	+	Radiation therapy. Died of metastases 2 yrs. after onset of symptoms
13. McFadzean (1)	29	+	+	0	+	Tumor excised 18 mos. after onset of symptoms. Follow-up restricted to 6 wks. after surgery
14. Östergaard (4)	28	+ (serotal)	+	+	0	Tumor excised 2 yrs. after onset of symptoms. No recurrence of symptoms or tumor 2 yrs. after surgery
15. Scott (1, 16)	43		+	+	+	Tumor excised 3 mos. after onset of symptoms. Died of metastases 9 mos. after onset of symptoms
16. Wilkins (1)	5	0	+	0		Tumor excised 4½ yrs. after onset of symptoms. No recurrence 4 yrs. after surgery
17. Armstrong & Simpson (5)	40	+	+	0		Tumor inoperable at exploration 6 mos. after onset of symptoms. Died of metastases 5 mos. after surgery
18. Staffieri *et al.* (6)	25	+	+	0		Tumor excised 2 mos. after onset of symptoms. Post-op. radiation. No recurrence 2 yrs. after surgery
19. Luft & Sjögren (16)	59	0	+	0		Tumor excised 5 mos. after onset of symptoms. Died on second postop. day
20. Mortensen & Murphy (7)	38	0	+	+	+	Tumor excised 18 mos. after onset of symptoms. No recurrence 5 mos. after surgery
21. Diczfalusy & Luft (8)	55	+	+			Tumor excised 1 yr. after onset of symptoms. Died of metastases 8 mos. after surgery
22. Myhre (9)	37	+	+	+	+	No treatment. Died of metastases 7 yrs. after onset of symptoms. Unrelated carcinoma of the prostate
23. Nusimovish (16)	40	+	+	+	+	Tumor inoperable 7 mos. after onset of symptoms. Died of cachexia after 1 yr. of symptoms
24. Pickard *et al.* (16)	14	+	+	+		Radiation therapy. Died of metastases 1 yr. after onset of symptoms
25. Curr & Vines (16)	47	+	+	+	+	Died during postop. period, 3 yrs. after onset of symptoms
26. Dohan *et al.* (10)	30	0	+	+	+	Tumor excised "a few months" after onset of symptoms. No recurrence 18 mos. after surgery
27. Dohan *et al.* (10)	58	+	+	+		Tumor excised 1 yr. after onset of symptoms. Died 6 mos. after surgery of unrelated malignant neurilemmoma. No evidence of recurrence of adrenal tumor at autopsy
28. Landau *et al.* (11)	28	0	+	+	0	Tumor excised 16 yrs. after onset of symptoms. No recurrence 1 yr. after surgery
29. Seror *et al.* (12)	56	+	+	0	+	Tumor excised 9 mos. after onset of symptoms. Follow-up restricted to 3 mos. after surgery
30. Staubitz *et al.* (13)	38	+	+	+	+	Alive with metastases 5¼ yrs. after onset of symptoms
31. Case Record, Mass. Gen. Hosp. (14)	66	+	+	0	+	Tumor incompletely excised 10 mos. after onset of symptoms. Recurrence 15 mos. after surgery. Died 3 yrs. after onset of symptoms
32. Dostal (15)						No further information available
33. Higgins *et al.* (16)	26	0	+	+	+	Tumor incompletely excised 10 mos. after onset of symptoms. Radiation & nitrogen mustard therapy. Died of metastases 16 mos. after onset of symptoms
34. Wallach *et al.* (present case)	28	0	+	+	+	Tumor excised 2 yrs. after onset of symptoms. Recurrence 2 yrs. after surgery

TABLE 2. ENDOCRINOLOGIC DATA IN 24 OF THE 34 REPORTED CASES OF FEMINIZING ADRENOCORTICAL CARCINOMA

Case report and reference*	Urine					Identification of urinary steroids	Remarks
	Chorionic Gonadotropin	Pituitary Gonadotropin†	Estrogen† (bioassay)	Androgen† (bioassay)	17-KS (mg./24 hrs.)		
6. Simpson & Joll (1)	Negative (A-Z test)		3200 m.u./24 hrs. ("Pathologic excess")	60 c.c.u./24 hrs. (Normal 8-25 c.c.u.)		Δ3-5, Androstandiene-17-one	Studies performed after excision of tumor but in presence of metastases
10. Cahill *et al.* (2)							Normal glucose tolerance, pigmentation, hypotension
11. Roholm & Teilum (1)	Negative (Friedman test)	<30 R.U./24 hrs. (Normal <30 R.U.)	5000 m.u./24 hrs. (Normal <20 m.u.)	164 c.c.u./24 hrs. (Normal 8-25 c.c.u.)			Normal glucose tolerance
12. Hurxthal & Musulin (3)			60 R.U./24 hrs. (Normal <10 R.U.)				
13. McFadzean (1)	Pos. (A-Z test)						Flat glucose tolerance curve
14. Öster gaard (4)	Negative (Friedman test)	<50 m.u./24 hrs.	200 m.u./24 hrs. ("Increased excretion")	25 I.U./24 hrs.			After excision of tumor, urinary pituitary gonadotropin, 50 m.u.; estrogen, 50 m.u.; androgen, 62 I.U./24 hrs.
15. Scott (1, 16)			5.3 μg. α-estradiol equiv./24 hrs. (Normal)		196.0	Dehydroisoandrosterone, 70 mg./24 hrs.	After excision of tumor, urinary 17-ketosteroid, 9.6 mg./24 hrs.; estrogen, 9.6 μg. α-estradiol equiv./24 hrs.
16. Wilkins (1)		<6.5 m.u./24 hrs.	5 R.U./24 hrs. "Slightly above (normal")		4.1		No urinary pregnanediol
17. Armstrong & Simpson (5)					34-108		
18. Staffieri *et al.* (6)					23.2		Severe reactive hypoglycemia

* Numbered according to the listing in Table 1.
† R.U. = Rat units.
m.u. = Mouse units.
I.U. = International units
c.c.u. = Capon-comb units.

(TABLE 2—*continued*)

Case report and reference*	Urine					Identification of urinary steroids	Remarks
	Chorionic Gonadotropin	Urinary Gonadotropin†	Estrogen† (bioassay)	Androgen† (bioassay)	17-KS (mg./24 hrs.)		
19. Luft & Sjögren (16)		<20 m.u./24 hrs.	250 I.U./24 hrs.		6.0		Hypertension, diminished glucose tolerance, insulin resistance. Bilat. adrenocortical hyperplasia & unilat. neoplasm
20. Mortensen & Murphy (7)	Neg. (A-Z test)				7.6		Flat glucose tolerance curve. Hyponatremia
21. Diczfalusy & Luft (8)						Free estriol, 34.5 μg./250 ml. urine Conjugated estriol, 857 μg./250 ml. urine Conjugated estrone, 68.5 μg./250 ml. urine Conjugated estradiol, 18.4 μg./250 ml. urine Pregnanediol, 29.0 mg./L.	
22. Myhre (9)	Negative (Friedman test)	<33 m.u./liter			256.0		Normal glucose tolerance
23. Nusimovish (16)		48–96 units‡/liter	5000 units‡/liter		6.9		Method and units not stated
24. Pickard, *et al.* (16)			160 I.U./24 hrs.		22–39	Pregnanediol, 49.5 mg./24 hrs.	Hypertension. Normal glucose tolerance. Urinary 11-oxysteroids, 26 mg./24hrs.
26. Dohan *et al.*, Case 1 (10)		24 m.u./24 hrs.	4000 m.u./24 hrs. (Normal 13-44 m.u.)		12.4		Hypertension. Normal glucose tolerance. Urinary corticoid, 4.0 mg./24 hrs. (normal, 2.0–5.0 mg./24 hrs.). After excision of tumor, hypertension relieved; urinary estrogen, 27 m.u./24 hrs.
27. Dohan *et al.*, Case 2 (10)			460 m.u./24 hrs. (Normal 13-44 m.u.)		5.8		Estrogen determined 2 days after excision of tumor; 6 mos. later urinary estrogen, 13 m.u./24 hrs.

‡ Normal range not stated.

(TABLE 2—*continued*)

Case report and reference*	Urine					Identification of urinary steroids	Remarks
	Chorionic Gonadotropin	Pituitary Gonadotropin†	Estrogen† (bioassay)	Androgen† (bioassay)	17-KS (mg./24 hrs.)		
28. Landau *et al.* (11)					30.0	HCl hydrolysis: Estriol, 57 μg./24 hrs. Estrone, 86 μg./24 hrs. Estradiol, 15 μg./24 hrs. Zn-HCl hydrolysis: Estriol, 74 μg./24 hrs. Estrone, 8 μg./24 hrs. Estradiol, 114 μg./24 hrs. Pregnanediol, 1.9 mg./24 hrs.	Normal results with water loading tes. Urinary formaldehydogenic cor.ic ids, 0.94 mg./24 hrs. (normal) After excision of tumor, urinary estriol, 13 μg./24 hrs.; estrone 9 μg./24 hrs.; estradiol, 10 μg./24 hrs. (HCl hydrolysis). Postop. urinary pregnanediol 0.03 mg./24 hrs.
29. Seror *et al.* (12)					9.2		
30. Staubitz, *et al.* (13)	Negative (A-Z test)	<3.2 m.u./24 hrs.	3340 I.U./24 hrs. (Normal 25–130 I.U.)		26–55		All data obtained after excision of tumor but in presence of metastases
31. Case record, Mass. Gen. Hosp. (14)	<8 m.u./100 cc. (neg.)	<6.5 m.u./24 hrs.	1235 μg. "estrogen" ("Normal 40–80 μg.)		510.0	Dehydroisoandrosterone, 300 mg. Etiocholanolone Androsterone 11-hydroxyandrosterone	After excision of tumor, urinary estrogen 80 μg./24 hrs. 17-ketosteroids, 16 mg./24 hrs. After recurrence, 17-ketosteroids, 38-45 mg./24 hrs.
33. Higgins, *et al.* (16)	Negative (Friedman test)	96 m.u./24 hrs.	2600–6200 μg./24 hrs.		150–600	Pregnanediol, 86.6–622.0 mg./24 hrs.	Normal glucose tolerance. Urinary 11-oxysteroids, 3.4 mg./24 hrs.‡ Analysis of tumor: estrogen (Kober reaction) 280 mg./70 Gm. of tumor tissue; pregnanediol (Guterman reaction), 4 mg./70 Gm. of tumor tissue; 17-ketosteroids, 3.1 mg./70 Gm. of tumor tissue
34. Wallach *et al.* (present case)	Negative (Friedman test)	0	73–177 μg. estrone equiv./24 hrs. (Normal 2–29 μg.)		8.6–13.2		Flat glucose tolerance curve; relative adrenocortical insufficiency. Progesterone, equilenin & 6 unidentified compounds isolated from tumor

amination. In one remarkable report (6), recurrent coma due to reactive hypoglycemia was the presenting symptom. In an occasional patient obesity, acne, hirsutism or pigmentation developed in association with feminization. In only 1 patient, that of Luft and Sjögren (cited in reference 16), were there more definitive stigmata of adrenocortical dysfunction. This patient manifested hypertension, diminished glucose tolerance and insulin resistance as well as the expected gynecomastia and testicular atrophy. Exploration revealed the unusual finding of bilateral adrenocortical hyperplasia and a unilateral adrenocortical tumor.

A flat glucose tolerance curve was noted in 3 of 9 patients tested. The present patient had a flat oral glucose tolerance curve and a feeble response to stimulation with corticotropin, suggesting the presence of relative adrenocortical insufficiency. The patient with reactive hypoglycemia, however, did not have a flat glucose tolerance curve.

The cellular pathology of the tumors of the reported cases was extremely variable. Many benign-appearing tumors developed widespread metastases, resulting in early death of the patient. Other tumors were clearly malignant by the usual criteria but appeared to carry a prognosis no worse than that of less anaplastic tumors. Inasmuch as metastases developed in 19 of the 34 patients cited, all adrenocortical tumors with gynecomastia should be considered malignant.

Endocrinologic aspects

The production of biologically active estrogens by these tumors has been well documented. Increased amounts of estrone, estriol, estradiol and pregnanediol have been found in the urine in 2 recent cases (8, 11). This suggests that the activity of many of these tumors is directed toward the production of steroids with benzenoid A rings (presumably estrogenic) and toward the production of progesterone. The identification of progesterone and some phenolic steroids with benzenoid A rings in the tumor of the present case strongly supports this view (17). The isolation from this tumor of equilenin (17), a weakly estrogenic steroid with benzenoid A and B rings, is of considerable interest, since this hormone has not been previously identified in man.

The laboratory evidence of adrenocortical hypofunction seen in some of these cases is most probably due to the suppression of pituitary production of corticotropin by one or more abnormal circulating steroids. As in the present case, this suppression persists for months after the tumor is removed. In the few instances in which Cushing-like characteristics have been described in addition to the feminization, the tumors were probably secreting steroids with 17-OHCS-like function as well as estrogens. In Luft and Sjögren's patient (16), the hyperplastic, nontumorous adrenal cortices

may have been the site of production of steroids with 17-OHCS-like action. The production of both estrogens and androgens by adrenocortical tumors has also been reported (1, 10). Feminization may not be present if androgen secretion predominates.

Small changes in the A ring and of the substituent groups on the perhydrocyclopentanophenanthrene nucleus have been found to result in great changes in physiologic action. It is reasonable to assume that the actions of the steroid hormones which produce feminization and masculinization may depend upon minor changes in the molecule. Scott's patient (1, 16) had gynecomastia and other evidence of feminization but there was no increase in urinary estrogen when measured by the usual bioassay methods. A greatly increased urinary 17-ketosteroid excretion was noted and large amounts of dehydroisoandrosterone, a metabolite of testosterone, were found. Dehydroisoandrosterone and related compounds have been found capable of inducing gynecomastia in laboratory animals (28), and the feeding of large amounts of testosterone to animals and man has resulted in increased urinary estrogens (29).

Cases of feminizing adrenocortical tumor in which elevated estrogen excretion was accompanied by very high 17-ketosteroid excretion or elevated titers of biologically active androgen, may not necessarily represent situations wherein two or more different types of steroids are being produced. It is possible that a steroid with one type of biologic and chemical property may undergo partial conversion in some other tissue to other steroids with distinctly different actions, resulting in a wide variety of biologic and chemical findings. Actual identification of the steroids in the tumor tissue and urine from patients with adrenocortical hyperplasia and neoplasms, and collateral studies of the biologic and chemical effects of pure steroids administered to human subjects and experimental animals are needed to elucidate this problem.

SUMMARY

1. The thirty-fourth reported case of a feminizing adrenocortical carcinoma is described. Gynecomastia was the presenting complaint in this patient.

2. Endocrinologic studies revealed elevated urinary excretion of estrogen, normal excretion of 17-ketosteroids, absence of both chorionic gonadotropin and follicle-stimulating hormone in the urine, a flat glucose tolerance curve, and a response to the corticotropin (ACTH) test which was indicative of relative adrenocortical insufficiency.

3. Pulmonary metastases developed two years after excision of the carcinoma. Prior to recurrence, all abnormal findings had regressed and the sperm count had risen from 1.7 to 26 million per ml.

4. The tumor contained moderate concentrations of progesterone and of equilenin, a weakly estrogenic steroid not previously found in human subjects. Six other unidentified steroids were also isolated (17).

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DETERMINATION OF Δ^4-3-KETO-CORTICOSTEROIDS IN HUMAN PERIPHERAL PLASMA BY THE ISONICOTINIC ACID HYDRAZIDE (INH) REACTION*

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THE purpose of this communication is to extend a previous report by the authors (1) on quantitative studies of various adrenocortical steroids in small samples of peripheral human blood plasma, by presenting the findings based on a new reaction (INH) which involves the formation of the isonicotinyl hydrazones of Δ^4-3-keto-corticosteroids. In conjunction with the Porter-Silber reaction (2) it provides an improved quantitative guide to the estimation of plasma corticosteroids. Actually, our application of this procedure has been part of an attempt to isolate and identify the various corticosteroids found in blood plasma.

Previous work. A new color reaction for the determination of Δ^4-3-keto-corticosteroids has recently been described by Umberger (3), who utilized the earlier findings by Ercoli, DeGiuseppe and De Ruggieri (4). When the isonicotinyl hydrazones of ketosteroids having a double bond conjugated with a carbonyl group are dissolved in acidified absolute ethyl alcohol, a yellow color (absorption maximum at 380 mμ) is produced. Under the conditions described by Umberger, this color is not produced by the hydrazones of the saturated ketosteroids or by those having a double bond in positions other than the alpha-beta position. The presence of the Δ^4-3-keto grouping is generally accepted as essential in all biologically active steroids. We have confirmed the claims of Umberger (3) for the specificity of this reaction and have further expanded these claims by demonstrating that there is no INH color reaction with allo-dihydro-hydrocortisone, a compound which contains the 3-keto group but not the double bond in the alpha-beta position.

METHODS

The experimental conditions of Umberger's method were adopted or only slightly modified for use on a micro scale. Due to the presence of variable amounts of INH-

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reactive materials in the organic solvents used in the various extractions and chromatographic procedures described by us (1), it was found necessary to adopt the alkaline blue tetrazolium treatment of the Mader-Buck reaction (1) for the methyl-alcohol and acetone solvents.

Reagents

#1. Blank reagent: 0.625 ml. of concentrated hydrochloric acid made up to a volume of 1000 ml. with freshly redistilled ethyl alcohol, absolute (not BT-treated).

#2. Isonicotinic acid hydrazide (INH)reagent: 25 mg. of isonicotinic acid hydrazide[1] (m.p. 172° C) dissolved in 50 ml. of Reagent #1.

Both reagents, when stored in the icebox, remain stable for several months.

Procedure

It is required that simultaneously with each set of unknown plasma samples a corresponding sample of distilled water is carried through the entire procedure in order to correct the final results for possible contamination by the solvents.

In general usage, *i.e.*, in determinations on both normal and abnormal plasma, it is suggested that a minimum of 20 ml. of plasma be employed for the performance of both the Porter-Silber and INH reactions. After carrying the plasma and water blanks through the various extraction and purification procedures described by the authors (1), each 15 per cent alcoholic chloroform eluate from the Florisil chromatographic column is split into 4 equal portions which are transferred into 4 glass-stoppered 40-ml. centrifuge tubes. The contents of the tubes are evaporated to dryness as previously described (1). To 4 of the dry centrifuge tubes (2 containing the solvent-blank eluates and 2 containing the plasma eluates) are added 0.5-ml. portions of reagents #1 and #2 respectively. All tubes are tightly stoppered and well mixed by rotation. After one hour standing at room temperature (not less than 25° C.) the contents of the tubes are transferred to 5 × 5 × 10-mm. microcuvettes and the optical densities of the plasma extracts are measured at 380 mμ in a Beckmann D.U. spectrophotometer, against the respective solvent blanks. The optical density of the plasma extract in reagent #1 is subtracted from that of the plasma extract in reagent #2. The corrected optical density is converted to micrograms of Δ^4-3-keto-corticosteroids contained in the amount of plasma represented by one of the 15 per cent alcoholic chloroform aliquots originally evaporated. A standard plot is prepared of optical density versus concentration, using the free alcohol of hydrocortisone at concentrations from 0.5 to 8.0 micrograms per 0.5 ml. of total volume.[2]

RESULTS

In Table 1 are shown the molecular extinction coefficients of 7 naturally occurring Δ^4-3-keto-corticosteroids and 1 synthetic androgen. It may be noted that these values are much more uniform than those obtained with

[1] Obtained from Taylor Chemical Company, St. Vincent & Sutherland Avenues, St. Louis 19, Missouri.

[2] For convenience of comparison and illustration of the sensitivity of this reaction, the result obtained at a concentration of 1.0 microgram of either corticosterone or hydrocortisone per 0.5 ml. of reaction mixture under the described conditions is 0.073 O.D.

It has been found with all compounds yet determined (Table 1) that this reaction strictly obeys the Beer-Lambert law.

TABLE 1. MOLECULAR EXTINCTION COEFFICIENTS

Compound* (free alcohol)	In methyl alcohol	INH reaction (380 mμ)
Cortisone, E_K	Max. 238 mμ, 15,000	10,400
Hydrocortisone, F_K**	Max. 242 mμ, 15,800	13,500
11-Desoxyhydrocortisone, S_R**	Max. 242 mμ, 15,000	11,400
11-Dihydrocorticosteroid, A_K	Max. 238 mμ, 16,500	11,300
Corticosterone, B_K	Max. 241 mμ, 16,700	12,500
11-Desoxycorticosterone, DOC Q_R	Max. 242 mμ, 16,500	12,100
Reichstein's substance E, Δ^4-pregnene-11,17,20,21-tetrol-3-one	Max. 241 mμ, 14,600	9,500
17-Ethyl-19-nortestosterone	Max. 241 mμ, 17,200	11,300

* Sources of materials are the same as described in reference 1, with the exception of 17-ethyl-19-nortestosterone, which was obtained through the courtesy of Dr. Robert Craig, G. D. Searle and Co., Chicago, Illinois.

** F_K = Kendall's compound F.
S_R = Reichstein's compound S.

the Mader-Buck and Gornall-MacDonald reactions (1), and agree very closely with certain molecular extinction coefficients given by Umberger (3).

In Table 2 are listed the data obtained in 20 samples of human plasma, all presumably "normal." It is seen that the range of the free INH values is from 6 to 24 micrograms per 100 ml. (average, 13.3; S.E., 1.0), whereas the range of Porter-Silber values is from 6 to 23 micrograms per 100 ml. (average, 12.9; S.E., 0.97). However, a comparison of the individual INH and Porter-Silber results for "free" steroids shows considerable variation in both directions. Among those showing Porter-Silber values higher than INH values is Patient #15, in whom the difference was as much as 16 micrograms per 100 ml. This suggests that, even in normal persons, much of the Porter-Silber reactive material may not be Δ^4-3-keto-corticosteroids, but may possibly be "free" tetrahydro E or tetrahydro F. In diseased states this difference is even more pronounced, as will be reported in detail subsequently (5). For example, in a severely burned patient the "free" INH-reactive material on successive days is (in micrograms per 100 ml. of plasma) 19, 46 and 42, as compared with Porter-Silber values of 42, 64 and 54 respectively.

As to the INH values which were greater than the Porter-Silber values (Table 2), the comparison between the former and the Mader-Buck alkaline blue tetrazolium reducing (BTR) materials ("free" alpha-ketol steroids) is of interest. In no case was the INH value higher than the BTR value; in fact, the BTR materials were generally present in much greater

TABLE 2. DATA ON "NORMAL" CONTROLS

Patient	Age & Sex	INH (Δ^4-3-keto-CS)		Porter-Silber (17-OHCS)		Mader-Buck (BTR equiv.)	
		Free (μg./100 ml.)	Conj.	Free (μg./100 ml.)	Conj.	Free (μg./100 ml.)	Conj.
1. C.L.	52, M	24	3	21	15	31	35
2. G.J.	28, M	19	4	14	20	23	111
3. M.M.	66, M	18	6	16	14	25	81
4. C.L.	52, M	18	8	16	9	25	29
5. R.S.	34, M	17	11	13	16	26	100
6. M.R.	38, M	15	13	9	28	21	89
7. D.K.	26, M	15	14	6	29	15	118
8. T.W.	48, M	14	3	15	11	15	104
9. B.D.	30, F	14	5	11	17	14	52
10. M.H.	36, M	12	4	9	6	19	62
11. H.D.	32, M	10	14	14	16	21	81
12. H.D.	32, M	8	13	13	22	17	68
13. F.S.	28, M	8	11	10	22	12	110
14. H.S.	16, M	7	21	13	13	34	59
15. L.A.	25, M	7	9	23	14	23	70
16. E.J.	32, M	6	18	16	20	30	81
17. Bank plasma	F	15	22	12	18	24	66
18. Bank plasma	M	15	22	10	20	21	66
19. Bank plasma	M	12	18	10	19	21	61
20. Bank plasma	F	13	17	6	17	15	56
Mean (micrograms/100 ml.)		13.3	11.8	12.9	17.3	21.6	75.0
S.E. (standard error)		1.05	1.44	0.97	1.26	1.33	5.52

amounts. We believe, therefore, that in the cases in which the "free" INH-reactive material was greater than the Porter-Silber reactive material, the difference could be accounted for by the presence of Δ^4-3-keto-corticosteroids which were not Porter-Silber reactive but were included in the BTR materials.

The values presented in Table 2 for the Δ^4-3-keto-corticosteroid conjugates (*i.e.*, INH-reactive materials which appear following beta-glucuronidase hydrolysis) suggest the possibility that corticosteroid conjugation with glucuronic acid may take place at carbon atom 21 without previous reduction at the Δ^4-3-keto grouping. This supposition is now being studied with techniques based on rigid methods of identification of these isolated materials. It will be noticed that the amounts of Porter-Silber reactive conjugates were generally greater than the amounts of INH-reactive conjugates, but that these values, even when added, were much lower than

those obtained with the BTR method following beta-glucuronidase hydrolysis.

In order to determine further the specificity of the INH reaction, we obtained blood samples at forty-five and seventy hours following withdrawal of hydrocortisone substitution therapy in a patient bilaterally adrenalectomized for carcinoma of the breast. *Zero* values were obtained for "free" Porter-Silber and INH-reactive materials in both of these plasma samples. These zero results are good evidence of the relative specificity of these two reactions.

In order to determine the replicability and duplicability of the INH reaction, we determined the 'free" Porter-Silber and INH-reactive materials in 5 aliquots of the same pooled "bank" plasma on two separate occasions. These results are presented in Table 3. We thought it also of interest to determine the 240 mμ absorption values on the methyl-alcohol solutions of the Florisil eluates of these same plasma samples. It will be noted in Table 3 that these values, calculated to hydrocortisone equivalents, were much higher and more variable than the corresponding INH values, due to the unavoidable presence of variable amounts of nonspecific ultraviolet-absorbing material derived from these partially purified plasma extracts.

The INH reagent (reagent #2) is very useful in various paper chromatography systems as a spray for the detection of Δ^4-3-keto-corticosteroids. We have determined experimentally that spots of certain adrenocortical steroids in amounts as low as 5 micrograms show up distinctly as bright yellow-green fluorescence under ultraviolet illumination. The papers are

TABLE 3. REPLICATE DETERMINATION OF STEROID LEVELS IN POOLED HUMAN PLASMA

Date	Sample (20-ml.)	Porter-Silber (17-OHCS) (μg./100 ml.)	INH (Δ^4-3-KCS) (μg./100 ml.)	240 mμ absorption (calc'd. to F_K* equiv.)
Dec. 6, '56	No. 1	10.8	13.0	26
	No. 2	10.5	11.8	35
	No. 3	10.3	12.0	34
	No. 4	10.0	12.0	30
	No. 5	11.8	11.8	38
Dec. 18, '56	No. 6	10.8	11.0	55
	No. 7	11.8	11.8	58
	No. 8	10.8	12.0	150
	No. 9	10.8	11.2	75
	No. 10	11.0	11.5	23

* Hydrocortisone.

simply sprayed with the reagent and allowed to dry for at least one hour at room temperature before observation. In our experience this reagent has proved to be superior to any of the commonly used methods for detection of adrenocortical steroids in paper chromatography.

COMMENT

The importance of the INH reaction as reported here stems from the fact that it may represent a measurement of the potentially biologically active steroids present in peripheral human plasma. Our findings, moreover, seem to indicate that the values obtained with the Porter-Silber reaction do not always represent "free" biologically active steroids, as shown by the normal findings listed in Table 2. This is particularly true in disease states.

The specific chemical estimation the Δ^4-3-keto-corticosteroids, in combination with the results obtained from the Porter-Silber reaction may further clarify the difficult problem of measuring metabolic variations in the glucuronic-acid conjugation of 17-hydroxycorticosteroids. The supposition that glucuronic-acid conjugation may take place at carbon atom 21 without previous reduction at the Δ^4-3-keto grouping is of obvious importance, because such a conjugate may itself possess biologic activity. We assume that the higher values obtained for Porter-Silber conjugates than for INH conjugates may possibly be explained by the presence of a greater amount of the glucuronic-acid conjugates of tetrahydro E and tetrahydro F.

These findings, if confirmed by isolation of "free" tetrahydro E and/or tetrahydro F, may prove of great practical importance in improving the means of correlating blood levels with adrenocortical activity and particularly with changes in the metabolic fate of secreted adrenocortical steroids.

SUMMARY

Findings with the isonicotinic acid hydrazide (INH) reaction as applied to human plasma suggest that this reaction differentiates between biologically active and inactive adrenocorticosteroids by chemical means.

These data used in conjunction with those obtained by the Porter-Silber reaction, both in health and disease, indicate that much of the "free" Porter-Silber material may exist in a biologically inactive form, presumably as tetrahydro E or tetrahydro F, or both.

Following beta-glucuronidase hydrolysis of human peripheral plasma, Δ^4-3-keto-corticosteroids appeared in considerable amounts, as measured by the INH reaction.

The possible significance of these findings for improved evaluation of adrenocortical activity is discussed.

The use of the INH reagent as a spray for the detection of Δ^4-3-keto-corticosteroids in paper chromatography is suggested.

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IN VIVO MEASUREMENT OF THYROIDAL RADIOIODINE: EFFECT OF "NECK SCATTER"

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THREE variables must be considered in any *in vivo* measurement of the content of radioiodine in the thyroid. The first is "neck scatter." The thyroidal component of neck scatter consists of the radiations that originate in the thyroid, initially directed outside the solid angle subtended by the counter, as defined by the collimation employed, and subsequently scattered into the counter by the tissues of the neck. The extrathyroidal component of neck scatter consists of those radiations which have a point of origin outside the angle subtended by the counter but which are scattered into the counter by the tissues of the neck.

The second variable is self-absorption. This includes those radiations originating in the thyroid, initially directed toward the counter, which are absorbed by photoelectric capture or are scattered away from the counter by the intervening tissues as a result of the well-known Compton effect.

The third variable is the distance from the skin to the effective center of radiation of the thyroid (1).

All three variables depend on the size of the thyroid gland, the size of the neck and the location of the gland in the neck. Since scatter, self-absorption and the distance of the effective center below the skin are presumed to be increased in larger necks, the augmentation of counting rates caused by scattered rays is somewhat counterbalanced in larger necks by the decreases produced by self-absorption and the greater distance from the counter to the thyroid, provided the counter is equally sensitive to all the radiations. Extrathyroidal scatter may be important when thyroidal uptake is low and the blood concentration of radioiodine is high.

Several authors (2–6) have given detailed attention to the problem of neck scatter. In a series of 25 patients whose thyroidal uptake was measured after the administration of I^{131}, Shipley and Clark (6) found that the contribution of scattered rays ranged from 14 to 46 per cent of the total activity, with a mean value of 25 per cent. On the other hand, Blom (2) used a fixed correction for back scatter of 21 per cent for all adults; he re-

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ported the individual variability to be only 5 per cent. This agrees with the work of Myant and associates (5), who took the actual value in the neck to be 79 per cent of the measured value and reported that the correction should vary from this value of 79 per cent by a standard deviation of not more than 3 per cent.

Since thallium-activated sodium iodide scintillation crystals have a relatively high sensitivity to the attenuated scattered rays, and since these crystals generally have supplanted GM tubes in the measurement of thyroidal uptake, the influence of scattered rays in the assay of uptake has become increasingly important.

One can discount scattered rays by applying a correction factor, as mentioned. One can interpose a 1.6-mm. lead filter between the neck and the crystal, as proposed by Shipley and Clark (6); although this technic effectively blocks the scattered rays, it also reduces the count of direct rays. One can purchase a spectrometer and measure selectively only the unattenuated gamma rays of I^{131}; as in the use of a lead filter, the measured radioactivity is reduced significantly in order to eliminate the weaker scattered rays. Manufacturers of scintillation spectrometers currently are stressing this method.

The present study was undertaken in an effort to determine whether the variability of scatter in measurements of thyroidal uptake by means of scintillation counters is sufficiently important to justify the decrease in counting rate and increase in expense that accompany differential counting methods.

MATERIALS AND METHODS

The counting equipment consisted of a thallium-activated sodium iodide crystal measuring 4.5 cm. in outside diameter and 5 cm. in depth that was connected optically to a Dumont type 6292 multiplier phototube. A cascaded voltage-regulator type "superstable" high-voltage supply was used to obtain dynode voltage. Pulses from the phototube were fed through a cathode follower to a linear amplifier, the output of which was connected in parallel to a discriminator and a pulse-height analyzer. Two scalers with a common preset time control were employed to totalize the counts from each of the two channels. All units except the scalers received input power from a line-voltage regulator (stabiline).

Figure 1 shows the spectra obtained with this system for a small source of I^{131}, with and without the presence of scattering material. These curves are similar to those published by other investigators (3–7).

In vivo measurement of thyroidal activity was made with the discriminator set at 0.05 million electron volts (mev). Counts in this channel thus represented both scattered and direct radiations. The pulse-height analyzer (PHA) accepted only pulses in the range between 0.30 and 0.41 mev. The PHA counts represented mainly the unscattered 0.364-mev photons that underwent photoelectric absorption in the crystal. The counter was positioned so that a distance of 20 cm. separated the front of the crystal and the anterior surface of the neck.

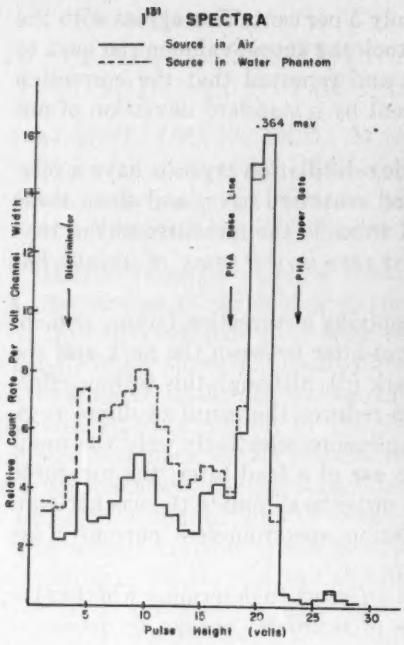

FIG. 1. Spectra of radioactivity of a small source of I^{131} measured by a pulse-height analyzer (PHA). The solid line represents a source in air and the broken line represents a source in a water phantom to produce scatter.

FIG. 2. Mock-up used to determine neck scatter. Note the posterior half of the lucite covering of Pandora. Human bones are used. The sponge-rubber lungs and wax larynx and trachea obscure the vertebral column. During use, the entire mock-up is filled with dry rice.

Calibration of the counter for I^{131} was accomplished by measuring daily the activity of one or more 15-ml. vials containing known amounts of I^{131}. The scattering medium in which the vials were placed for these measurements was a Pyrex glass baking dish measuring 7.5 by 11.5 by 20 cm. and containing water to a depth of 5 cm., together with an aluminum "doughnut." The bottle was placed horizontally on the doughnut, with its upper edge 1.6 mm. below the surface of the water. This convenient "mock-up" was selected for daily calibration after its spectrum of scatter had been found to be the same as that of a more authentic phantom, named Pandora.[1] Pandora's upper torso, neck and head consisted of a molded plastic shell containing human bones and filled with rice, the scatter of which closely simulates that of human tissues (Fig. 2). Molded hollow latex "thyroid glands" were filled with standard quantities of I^{131}, placed on a wax trachea and inserted into Pandora's neck (Fig. 3). The channel ratio, namely discrimination counts divided by the PHA counts, was found to be the same for Pandora and the glass-dish mock-up.

[1] Pandora was designed and constructed at the Mayo Foundation Museum of Hygiene and Medicine under the direction of Dr. A. H. Bulbulian.

FIG. 3. Three different artificial thyroids are shown, one viewed posteriorly. Note the openings at the top through which radioiodine is placed into the "thyroid."

The location of the 0.364-mev photoelectric peak in the spectrum of I^{131} was checked several times each week to insure that the over-all gain of the counter and amplifier had not shifted appreciably. Gain of the amplifier was adjusted if this peak had moved from its average position by more than 5 per cent. Lowering the limits on this fluctuation would have required more detailed spectra with narrower channel width and was not deemed to be practical, since this study was designed to test the importance of scatter under conditions similar to those in a clinical laboratory engaged in the measurement of thyroidal uptake of I^{131}. Such a maximal shift in gain would change the discriminator counts by ± 0.5 per cent and the PHA counts by ± 4 per cent for the settings used.

Day-to-day fluctuations in the sensitivity of one channel relative to the other, presumably caused by this allowable variation of over-all gain, are shown in Figure 4. In this diagram, the activity in the mock-up, as measured by each of the channels, is plotted with the PHA assay as the abscissa and the discriminator value as the ordinate. One would expect all the points to fall along the straight line shown if no statistical fluctuations or changes in sensitivity occurred.

Simultaneous PHA and discriminator measurements of thyroidal uptake were made on 140 patients who had been given routine diagnostic tracers containing 5 microcuries of I^{131} (a few patients received doses of 25 microcuries when clearance studies were desired). The readings were taken at six or at twenty-four hours after a !ministration of the dose, or at both times. Triplicate counts for periods of 100 seconds were made in each instance, with the sensitivity of the counter being 40 counts per second per microcurie in the PHA channel and 115 counts per second per microcurie in the discriminator channel.

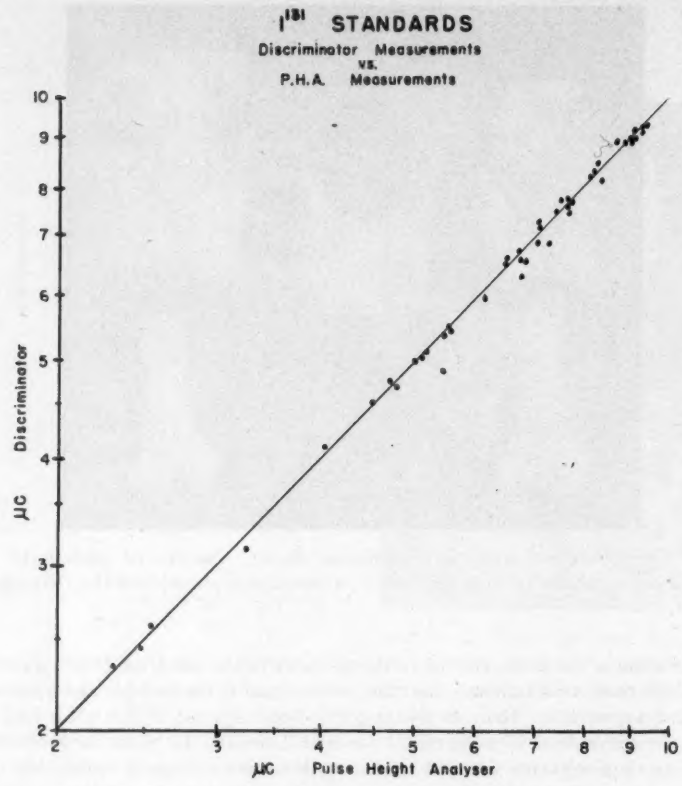

FIG. 4. Day-to-day variations in discriminator and PHA measurements of radioactivity from a mock-up containing I^{131} standards.

RESULTS

The results of the *in vivo* measurements are presented in Figures 5 and 6.

Figure 5 shows the 6-hour thyroidal uptake, calculated in microcuries, as measured in the discriminator channel plotted against the value obtained in the PHA channel. Points representing uptakes greater than 1.0 microcurie were generally within ± 5 per cent of the line of equality. With values less than 0.5 microcurie, the discriminator readings were consistently greater than the corresponding PHA measurements.

The data for the 24-hour uptake appear in Figure 6. With values more than 1.0 microcurie, the points were distributed about the line of equality in much the same manner as they were for 6-hour uptakes. However, the preponderance of large discriminator:PHA ratios with values less than 0.5 microcurie seen in Figure 5 practically disappeared at twenty-four hours.

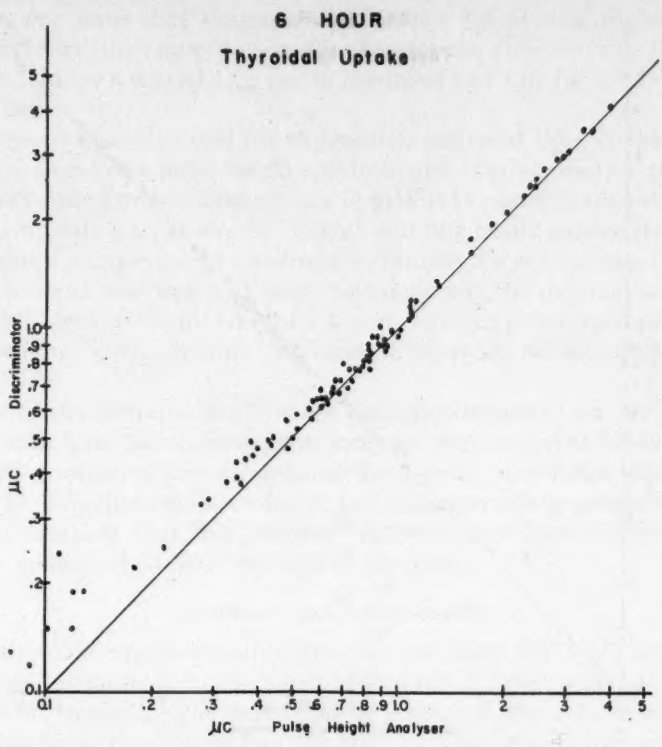

FIG. 5. Discriminator and PHA measurements of the 6-hour thyroidal uptake of radioiodine in patients receiving tracer doses of I^{131}. Compare the distribution of these values with the standards seen in Figure 4.

COMMENT

Comparison of these *in vivo* data with the standardization measurements (Fig. 4) shows that the errors introduced by the variability of neck scatter are no greater than those introduced by instrumental fluctuations under reasonably controlled clinical conditions when the thyroidal uptake is greater than 20 per cent of the dose of I^{131}.

The difference that exists between the discriminator and PHA measurements with values less than 1.0 microcurie, particularly those less than 0.5 microcurie, at six hours must be attributed to scatter. However, the fact that these differences largely have disappeared at twenty-four hours, coupled with the fact that the differences are greater for small uptake values than for larger readings, points to the likelihood that the extrathyroidal component of neck scatter is the one primarily involved. This type

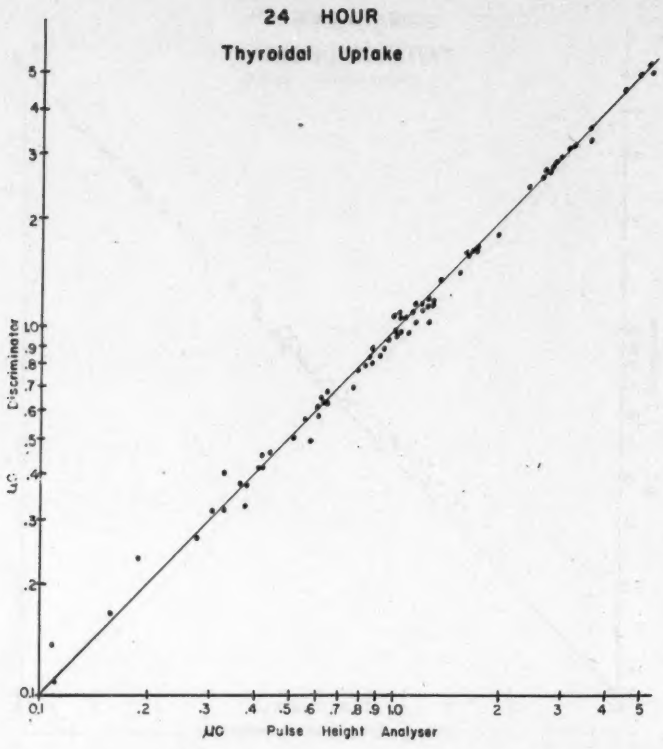

FIG. 6. Discriminator and PHA measurements of the 24-hour thyroidal uptake of radioiodine in patients receiving tracer doses of I^{131}. Compare with the standard in Figure 4.

of scatter comes mainly from the radioiodine circulating in the blood, and it would be expected to decrease in relative importance with time as the radioactivity is cleared from the blood by the thyroid and kidneys, especially by the latter when thyroidal uptake is small. The relative importance of the thyroidal component of scatter depends only on the physical characteristics of the neck being measured and not on the time of measurement or amount of uptake.

The relative importance of back scatter in any given measurement is influenced by the size of the crystal used in the scintillation counter and becomes less significant with larger crystals. Berger and Doggett (8) calculated the efficiency for various thicknesses of sodium iodide crystals at certain photon energies. From their data and from similar calculations of efficiency at lower energy based on the published (9) sodium iodide cross-

section, one finds that the ratio of efficiency for rays of the energy of scattered rays (0.15 mev) to the efficiency for the direct rays of I^{131} (0.364 mev) is 1.74 for a crystal 1.25 cm. in thickness and 1.07 for a crystal 5 cm. in thickness.

It appears that the need for eliminating scattered rays, either by lead filtration or use of a pulse-height spectrometer, is minimized by the use of larger crystals. Further, fluctuations in gain that would change the counting rate by only 0.5 per cent in integral counting would change the rate by 4 per cent if a pulse-height spectrometer limited the rays to those with energies between 0.30 and 0.41 mev. Not only was the differential counter potentially less stable in our study but the counting rate was reduced almost two thirds, namely from 115 counts to 40 counts per second per microcurie of I^{131}.

None of the discrepancies between the measurements from the discriminator and from the pulse-height analyzer demonstrated in this study would have impaired the usefulness of the tests for our routine clinical purposes (10, 11), since the need for precise measurement is primarily in the range of uptakes that fall between euthyroid and hyperthyroid zones, namely uptakes of 35 to 55 per cent of the dose.

SUMMARY AND CONCLUSIONS

The thyroidal uptake of radioiodine was measured at the Mayo Clinic in a series of 140 patients by the simultaneous use of differential and integral technics of counting. The variability of scatter of the rays is important only when measurements within the first few hours are desired, and when it is necessary to measure small uptakes accurately for special clinical studies. Integral counting without rejection of the scattered rays is adequate for routine measurement of thyroidal uptake. In fact, it is to be preferred to differential counting because of the greater stability of calibration with respect to fluctuations in gain and because of the larger counts associated with the integral method, which permits the use of smaller doses of I^{131}.

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METABOLIC EFFECTS OF 17-ETHYL-19-NORTESTOSTERONE IN MAN*

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TESTOSTERONE is widely used in the treatment of osteoporosis (1, 2) and of metastatic breast carcinoma (3). Improvement in well-being, appetite and general strength, decrease of bone pain, weight gain, recalcification of osteolytic lesions and shrinkage of soft-tissue metastases have been noted. However, hirsutism, acne, change of voice and of libido are disturbing side-reactions. In addition, the hypercalcemic syndrome has been observed in 10–15 per cent of patients with metastatic breast carcinoma who were treated with testosterone; this toxic side-reaction was thought to be due to stimulation of growth of the hormonally dependent tumor (4, 5). In view of these undesirable effects, several testosterone compounds have been assayed in the hope of finding a potent anabolic agent less androgenic than testosterone (6–8). The testosterone derivative 17-ethyl-19-nortestosterone (Nilevar[1]) was shown to have the greatest separation of androgenic and anabolic effects in rats (9, 10). The favorable ratio of anabolic to androgenic potency in experimental animals suggested the metabolic evaluation of this compound in man. Therefore, Nilevar was administered intramuscularly to 5 patients whose dietary intake was controlled on the metabolic research ward (11). The effects of this agent on protein and mineral metabolism were studied under this experimental arrangement, but no evaluation was made of androgenic potency.

MATERIALS AND METHODS

The age and sex of the patients, the diagnoses, the duration and the type of the studies, and the dosages of the compound are listed in Table 1. All patients were ambulatory and received a controlled, analyzed, low-calcium diet containing, 1,950 calories per day (67 Gm. of protein, 267 Gm. of carbohydrate and 69 Gm. of fat) and approximately 160 mg. of calcium and 760 mg. of phosphorus per day (12). The fluid intake was kept constant. The study was divided into three phases: 1) the control phase (fourteen to

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† Research Fellow, Dazian Foundation for Medical Research.

[1] The authors wish to thank Drs. Irwin C. Winter and Robert L. Craig, of G. D. Searle & Co., Chicago, for their kind cooperation.

twenty-four days), 2) the treatment phase (ten to forty-eight days) in which a dose of 25 or 50 mg. of the compound was administered intramuscularly daily, and 3) the post-treatment phase (twenty-two to twenty-four days). Patient 3 did not agree to stay at the hospital for the post-treatment study. The urinary nitrogen, phosphorus and creatinine, the fluid intake and output, and body weight were determined daily during the three phases of the study. Metabolic balances of nitrogen, phosphorus, calcium, potassium and sodium were determined on six-day pools of urine and feces. Calcium tolerance tests were performed prior to, and at the end of the treatment phase in all patients (13–15).

The nitrogen, calcium, phosphorus, sodium and potassium content of the food was determined on aliquots of the diet of each six-day metabolic period. The urinary and fecal nitrogen excretions were determined by the Kjeldahl method, and phosphorus excretion by the method of Fiske and SubbaRow (16). The urinary calcium was measured by the method of Shohl and Pedley (17), and the calcium content of the stool and urine pools was determined on dried acid-ashed aliquots of six-day metabolic pool collections. Urinary and fecal sodium and potassium excretions were determined by the flame-photometer method on aliquots of six-day pools.

Another parameter was employed to study the effects of Nilevar on skeletal metabolism in Patients 2 and 3. In the pre-treatment phase, tracer doses of 0.14 μc. and 0.26 μc. of radiostrontium (Sr^{85}) per Kg. of body weight were injected intravenously into the respective fasting patients (18–20). Sr^{85} emits pure gamma rays with a 65-day half-life. Plasma levels, and urinary and fecal excretions of Sr^{85} were determined daily for fifteen days and the strontium retention was calculated.[2] Second tracer doses of Sr^{85} (0.17 μc. and 0.13 μc. per Kg. of body weight, respectively) were injected into the same patients on the twenty-fifth and twenty-first days of treatment, and the foregoing analyses again performed (see Fig. 3 and text). A third tracer (0.27 μc. per Kg. of body weight) was injected into Patient 2 on the forty-third day of administration of Nilevar.

RESULTS

A dose of 25 mg. of 17-ethyl-19-nortestosterone (Nilevar) administered daily for eighteen days had no appreciable clinical or metabolic effect in 2 patients (Nos. 4 and 5, Table 1); the balance data on these 2 patients are, therefore, not listed in Table 2 among the data on the other 3 patients. However, when a dose of 50 mg. was injected daily, the urinary nitrogen and phosphorus excretion decreased significantly on the first day of the administration of the compound.

Figure 1 shows the results in a 58-year-old ambulatory female patient (No. 1) with mild osteoporosis, who received 50 mg. per day for eighteen days. There was a considerable decrease in the urinary excretion of nitrogen on the first day of the injection of Nilevar. Although retention of nitrogen persisted throughout the entire treatment phase and for four days thereafter, a tendency toward a gradual lessening of effectiveness is noted. The rebound phenomenon is clearly seen in the last two periods after discontinuation of treatment. Concomitant with the decrease of urinary nitro-

[2] Carrier-free Sr^{85} was obtained from the Nuclear Science and Engineering Corporation, Pittsburgh, Pa.

TABLE 1. DATA ON 5 PATIENTS RECEIVING 17-ETHYL-19-NORTESTOSTERONE INTRAMUSCULARLY

Patient No.	Age (yrs.) & Sex	Diagnosis	Dosage (mg./day)	Duration of therapy (days)	Total dose	Type of study	Duration of metabolic study (days)	Clinical observations	Side reactions
1	58 F	Mild osteoporosis	50	18	900	Metabolic balances; calcium tolerance tests	64	Increased appetite; weight gain	None
2	35 M	Carcinoma of nasopharynx	50	48	2,400	Metabolic balances; calcium tolerance tests; Sr^{85} intravenously	86	Increased appetite; weight gain	None
3	71 F	Senile osteoporosis	50	24	1,200	Metabolic balances; calcium tolerance tests; Sr^{85} intravenously	48	Increased appetite; weight gain	None
4	60 M	Pulmonary emphysema and fibrosis	25	10	250	Metabolic balances; calcium tolerance tests	30	No change	None
5	71 F	Senile osteoporosis	25	10	250	Metabolic balances; calcium tolerance tests	30	No change	None

gen, urinary phosphorus decreased from an average daily excretion of 484 ± 18 mg. to an average of 228 ± 39 mg. in the first treatment period. This retention continued during therapy and for four days after discontinuation of the compound. Phosphorus excretion then returned to pre-treatment levels, and even exceeded them in the following twelve days. There was a slight gain in body weight during treatment and a progressive decrease after discontinuation of therapy.

Figure 2 shows the prompt and distinct decrease in urinary nitrogen and phosphorus excretion in a 71-year-old ambulatory female patient (No. 3) with senile osteoporosis, who received 50 mg. of Nilevar per day for twenty-four days. The urinary nitrogen decreased from a daily average of 7,837 ± 194 mg. prior to treatment, to an average of $6,783 \pm 371$ mg. during the first six-day period of treatment; at the same time the urinary phosphorus decreased from a daily average of 496 ± 22 mg. to an average of 353 ± 23 mg. The decrease in urinary nitrogen and phosphorus persisted for the entire treatment phase. The increase of body weight in this patient was more marked than in Patient No. 1. The urinary calcium decreased slightly during treatment. The calcium tolerance test (13–15) was performed prior to, and at the end of three weeks of hormone administration and the data are shown in Figure 2. Calcium retention was 51 per cent in Test I, whereas it was 67 per cent in Test II.

Figure 3 shows the rate of excretion and the cumulative amount of radioactive strontium (Sr^{85}) in the urine of Patient 3 before and during therapy.

TABLE 2. METABOLIC BALANCES IN 3 PATIENTS RECEIVING 17-ETHYL-19-NORTESTOSTERONE*

Days	Treatment	Body Weight kg	FLUID, ml/day			NITROGEN, mg/day				PHOSPHORUS, mg/day					CALCIUM, mg/day				POTASSIUM, mEq/day			
													Balance									
			Intake	Urine	Bal.	Intake	Urine	Stool	Bal.	Intake	Urine	Stool	Act.	Theor.	Intake	Urine	Stool	Bal.	Intake	Urine	Stool	Bal.
PATIENT 1																						
24	none	73.5	2675	1981	+ 694	10630	8701	1088	+ 841	690	503	189	− 2	+ 40	153	48	143	− 38	55.2	41.1	6.8	+ 1.7†
12	17-ethyl-19-nor-testosterone‡	73.5	2690	1558	+1132	10129	6335	899	+2895	674	223	172	+279	+200	147	30	111	+ 6	48.9	29.3	3.9	+15.7
22	none	73.4	2681	1641	+1040	10551	8475	1004	+1072	694	484	160	+ 69	+ 73	133	37	97	− 1	49.6	42.8	7.7	− 0.9
PATIENT 2																						
14	none	45.5	2555	1942	+ 613	10410	8522	1056	+ 802	706	467	155	+ 84	+ 7	131	103	133	−105	49.6	47.1	6.4	− 3.9
42	17-ethyl-19-nor-testosterone‡	48.6	3605	2665	+ 940	10881	7531	949	+2401	731	364	158	+294	+145	140	68	112	− 40	50.5	33.7	6.0	+ 10.8
24	none	51.2	3876	3044	+ 836	10896	9801	1138	− 73	729	668	239	−178	− 77	145	81	222	−158	50.9	48.7	7.8	− 5.6
PATIENT 3																						
24	none	39.9	2722	1541	+1181	10530	8283	970	+1277	690	467	132	+ 91	+ 67	144	117	72	− 44	49.6	39.5	7.0	+ 3.1
24	17-ethyl-19-nor-testosterone‡	41.1	2720	1218	+1502	10463	6444	724	+3295	681	330	114	+236	+232	135	70	48	+ 17	49.6	35.2	5.2	+ 9.2

* Nilevar (G. D. Searle & Co.).
† Average of the 6-day metabolic period prior to the administration of the hormone.
‡ Dosage—50 mg. intramuscularly daily.

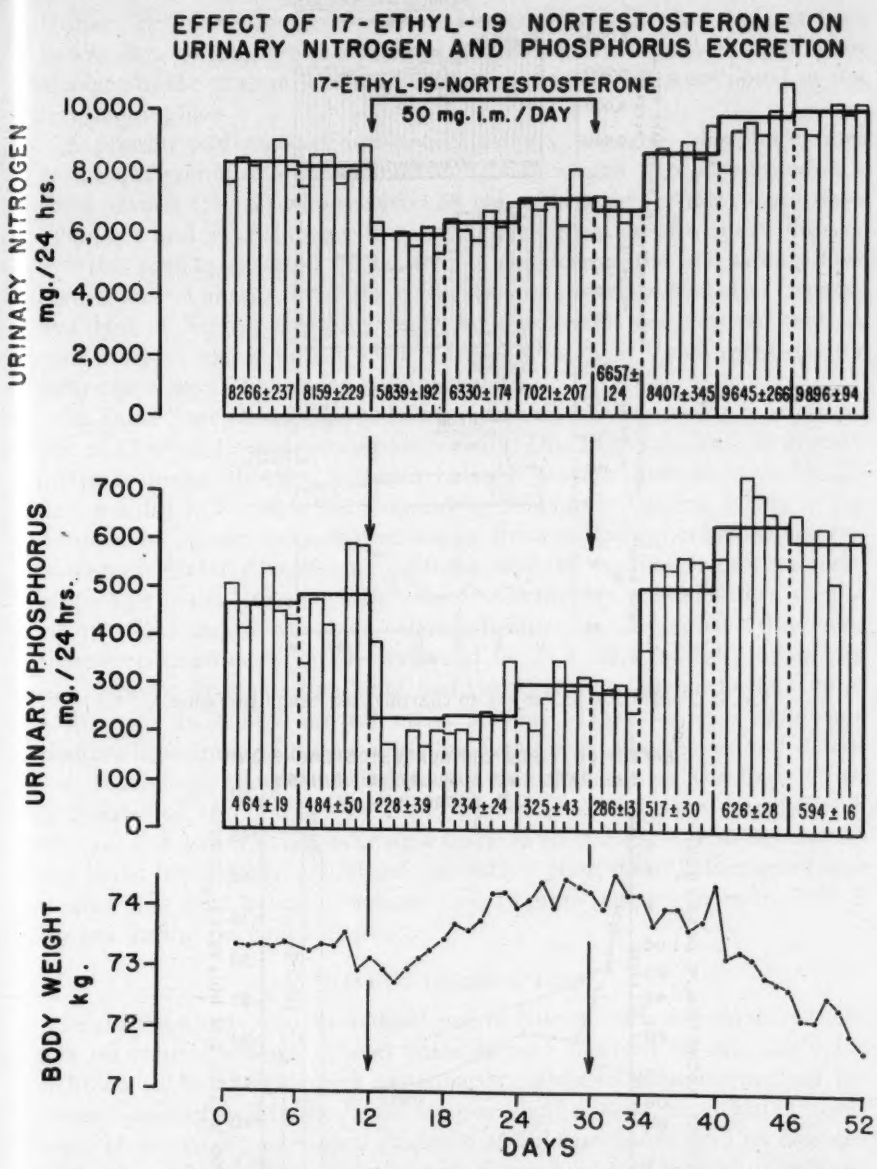

FIG. 1. *Patient No. 1.* The narrow columns represent the daily urinary nitrogen and phosphorus excretions. The averages with the standard errors for the respective metabolic periods are entered at the bottom of each group of columns, and are represented by the heavy lines near the top.

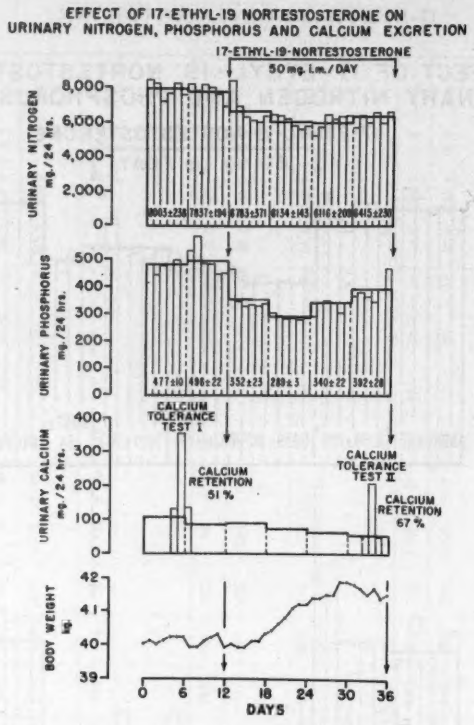

FIG. 2. *Patient No. 3.* For key to charting, see legend to Figure 1.

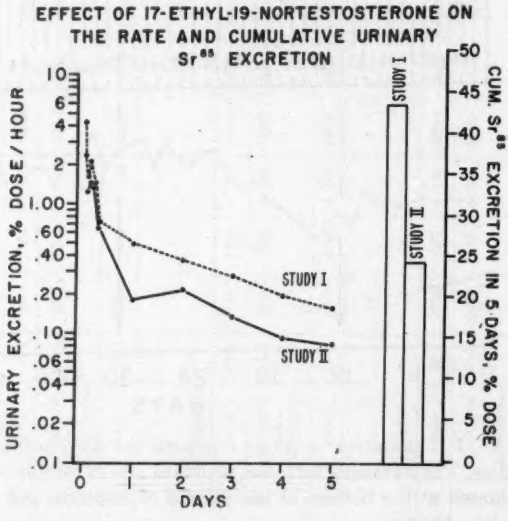

FIG. 3. *Patient No. 3.* Study I represents the pre-treatment phase. Study II was started on the twenty-first day of therapy.

Urinary Sr^{85} excretion was distinctly lower during therapy—24 per cent in five days as compared to 43.5 per cent before treatment. No significant changes in the plasma level and fecal excretion of Sr^{85} were noted in the treatment phase.

A prompt and marked decrease of urinary nitrogen, phosphorus and potassium excretion and an increase in body weight were also noted in a third patient (No. 2) who received 50 mg. of Nilevar for forty-eight days (Tables 1 and 2). Following the intravenous injection of a tracer dose of Sr^{85} this patient excreted 17 per cent of the dose in the first twenty-four hours (control phase); when the tracer dose was administered after twenty-five days of Nilevar therapy, the 24-hour excretion was 12.5 per cent. A similar result was obtained when a tracer dose of Sr^{85} was injected after forty-three days of hormone administration.

In Table 2 are the metabolic balance data on 3 patients who received 50 mg. of 17-ethyl-19-nortestosterone per day. Due to the decrease in urinary nitrogen during therapy, nitrogen balance became more positive by 2.0 Gm. per day in Patients 1 and 3, and by 1.6 Gm. in Patient 2. Due to the decrease in urinary phosphorus during therapy, phosphorus balance became more positive by 281 mg., 210 mg. and 145 mg. per day respectively in the 3 patients. Urinary calcium decreased slightly or moderately, resulting in slight improvement of calcium balance in the positive direction. Urinary potassium excretion decreased by 11.8, 13.4, and 4.3 mEq. per day respectively in the 3 patients, and the potassium balance became more positive by 14.0, 14.7 and 6.1 mEq. per day. There were no significant changes in sodium excretion, and therefore these balances are not listed. The theoretical phosphorus balances were calculated on the basis of an N:P ratio of 14.7:1.0 in "average" protoplasm, and were corrected for changes in calcium balances using a Ca:P ratio of 2.2:1.0. Fair agreement was noted between the actual and theoretical phosphorus balances in these studies. The fluid balances became slightly more positive in each of the 3 studies during hormonal therapy.

CLINICAL OBSERVATIONS

In the 2 patients who received 25 mg. of Nilevar daily for ten days there was no clinical change. The 3 patients who received 50 mg. daily for eighteen to forty-eight days experienced increased appetite and an increased sense of well-being. There were no signs of acne, hirsutism or deepening of the voice; no clinical evidence of fluid retention; and no changes in blood pressure, pulse or temperature. Complete blood counts, urinalyses, and determinations of the levels of blood urea nitrogen and serum sodium, potassium, calcium, phosphorus and alkaline phosphatase were made once weekly; no changes were noted during hormonal therapy.

DISCUSSION

The androgenic and anabolic effects of various testosterone derivatives were recently investigated in animals by Drill and Saunders (9, 10). These authors concluded that 17-ethyl-19-nortestosterone is the most promising testosterone derivative, since it is a potent anabolic agent which also has the most favorable anabolic/androgenic ratio and is orally much more potent than methyltestosterone or testosterone propionate. Since such a compound is of potential interest for clinical therapeutics, application of these data obtained in animals was warranted in man.

In our series of patients, the anabolic activity of 17-ethyl-19-nortestosterone was indicated by a marked decrease in urinary nitrogen, phosphorus and potassium excretions, and a consequent increase in the positive balances of these constituents. Calculation of the theoretical phosphorus balances and the comparison of the theoretical and actual phosphorus balances indicated new formation of an "average" tissue protein. Administration of the hormone did not cause any appreciable retention of salt and water. Therefore, the expectations based on animal studies were confirmed in man.

The effectiveness of this compound upon calcium metabolism warrants further comment. In none of the 5 patients was there evidence of major calcium loss prior to therapy and therefore no major improvement of calcium metabolism was expected with therapy. However, there was a slight decrease of urinary calcium excretion in each of the 3 patients who received the higher dose of Nilevar. In Patient 3, a 71-year-old female with senile osteoporosis, marked improvement was noted in the results of the calcium tolerance test; calcium retention rose from a low pre-treatment value of 51 per cent to a normal level of 67 per cent after treatment. This improvement is similar to that previously described in patients with osteoporosis treated with steroid hormones (21). The improvement of strontium metabolism noted in the same patient is further evidence of the change in mineral metabolism secondary to hormonal therapy. The mechanism of the action of this and of related compounds upon calcium and strontium metabolism in normal subjects and in various disease states is currently being investigated at this institution. The decreased urinary calcium and strontium excretion without significant changes in plasma levels, and the increased retention of intravenously administered calcium following steroid therapy indicate dual action upon kidney clearance and bone metabolism.

Although these studies attest to the anabolic action of 17-ethyl-19-nortestosterone in man, the evaluation of androgenic action, toxicity and palliative effectiveness in metastatic mammary carcinoma requires prolonged administration of this compound. In regard to its androgenic po-

tency, Escher (22), Gordan (23), and Weston (24) commented on the weak androgenic action of Nilevar in man. Heller found Nilevar to be less androgenic than testosterone propionate in eunuchs (25). Herrmann *et al.* administered at this institution a total of 3,000 mg. of Nilevar intramuscularly over a period of three months to 17 female patients with metastatic carcinoma of the breast (26). In these patients signs of virilization were distinctly less than in patients receiving comparable dosages of testosterone propionate over the same period of time.

SUMMARY

The metabolic effects of 17-ethyl-19-nortestosterone have been studied in 5 patients. A daily intramuscular dose of 50 mg. resulted in a prompt and significant decrease in urinary nitrogen, phosphorus and potassium excretions and increased positive metabolic balances of these constituents. There was also an increase in body weight. The anabolic effect lasted for a few days after discontinuation of therapy. No significant retention of salt or water was noted during treatment. There was some improvement in calcium metabolism, particularly in a patient with senile osteoporosis, as judged by results of the calcium tolerance test and by radiostrontium studies.

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TURNER'S SYNDROME AND LAURENCE-MOON-BIEDL SYNDROME IN SIBLINGS

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INTRODUCTION

TURNER'S syndrome (1) and Laurence-Moon-Biedl syndrome (2) are rare conditions, each of which is associated with multiple genetic defects. The Laurence-Moon-Biedl syndrome often occurs familially (3); Turner's syndrome rarely does. We believe that this is the first report of the occurrence in siblings of the two syndromes separately—typical Turner's syndrome in a woman and Laurence-Moon-Biedl syndrome in her brother.

CASE REPORTS

Turner's syndrome

A 31-year-old woman was initially examined here in April 1947, because of her unusually short stature and because she had never menstruated. Her birth and early development apparently had been normal, but physical growth stopped at the age of 11 years. When she was 12 years of age the right eye became affected by an inflammatory disease that resulted in ocular proptosis that failed to regress. Menarche had not occurred and there was no evidence of breast enlargement or of other secondary sexual characteristics. However, she had been active and vigorous and generally felt well.

One of the patient's 4 siblings, a brother, was blind, mentally deficient, and is described subsequently. The other 3 siblings, the parents, and other relatives were apparently normal.

Physical findings. The patient was 55 inches in height and weighed 91 pounds (Fig. 1). Despite her short stature, the body habitus was muscular and well developed. The thorax was full, with moderate pectus excavatum. There was mild webbing of the neck, prominent cubitus valgus, and slight webbing of the toes. Both wrists flared, principally from enlargement of the distal ulna. Axillary and pubic hair was abnormally sparse. There was no discernible development of mammary tissue, and the genitalia were infantile. Speculum examination revealed an infantile cervix. The uterus was small but could be probed to a depth of 4 cm. Ovaries were not palpable.

There was proptosis of the right eye and slight ptosis of the upper lid. Upon measurement with an exophthalmometer, the anteroposterior reading for the right eye was 23 mm. and for the left eye, 19 mm. A grade II blowing diastolic murmur was audible at the cardiac base to the right of the sternum; the pulmonic second sound was accentuated. The blood pressure was 120/78 mm. Hg.

Laboratory findings. Hemoglobin concentration, 12.5 Gm. per 100 ml.; leukocyte count, 6,300 per cu. mm.; results of urinalysis, normal; fasting blood sugar level, 84 mg. per 100 ml.; basal metabolic rate, +9 per cent; urinary gonadotropins, more than 424

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mouse units (4); and 17-ketosteroid excretion, 2.8 mg. per twenty-four hours (5). An endometrial biopsy was attempted but produced insufficient tissue for diagnostic purposes. The cells in a vaginal smear were completely castrate in type.

X-ray findings. The bones of the calvaria and the sella turcica were normal. Minimal lateral bowing was evident in the lower third of the radii, and there was some demineralization of the bones of the hands and wrists.

FIG. 1. Turner's syndrome in a 31-year-old woman. Note short stature, relative infantilism, and mild webbing of the neck.

Treatment and results. The patient took stilbestrol for several weeks but discontinued it because she became nauseated. No further estrogen therapy was attempted.

She was re-examined in May 1956, nine years after initial examination. There was no significant change in her physical condition. She had been working regularly and was without complaint except for episodes of nervousness. The findings on physical examination were unchanged, except that the basal diastolic murmur was softer and more blowing in quality. A roentgenogram of the chest and an electrocardiogram were normal. Studies of the leukocytes and a skin biopsy for genetic sex determination indicated genetic male sex.

Laurence-Moon-Biedl syndrome

The 29-year-old brother of the patient just described was initially examined here in April 1947. At birth he had a supernumerary finger and a supernumerary toe bilaterally. During infancy and early childhood, there were delayed dentition and pronounced retardation in development of motor co-ordination. When he was less than 1 year old, he became obese, and it was evident that he was mentally retarded. Impairment of vision, first noted when he was 5 years of age, progressed, and at the time of examination he was almost totally blind. Nine years before examination, he began to have episodes of mental depression followed by periods of agitation and delirium which necessitated his commitment to a state mental hospital.

Physical findings. The patient was a moderately obese man, 67 inches tall (Fig. 2A), with a blood pressure of 118/98 mm. Hg. He had an awkward, somewhat ataxic gait. There were coarse nystagmoid movements of the eyes that manifested light perception only. There was anisocoria, and funduscopic examination revealed scattered retinal pigmentation. Each foot had 6 toes (Fig. 2B), and each hand had a scar where an extra

digit had been surgically removed. A short, moderately loud systolic murmur was present to the left of the sternum. The penis was underdeveloped, measuring $5\frac{1}{2}$ cm. from the base (as close to the pubis as pressure would permit) to the tip; however, testicular size (4 cm. by 3 cm.) appeared to be normal, and the prostate was normal on palpation. He

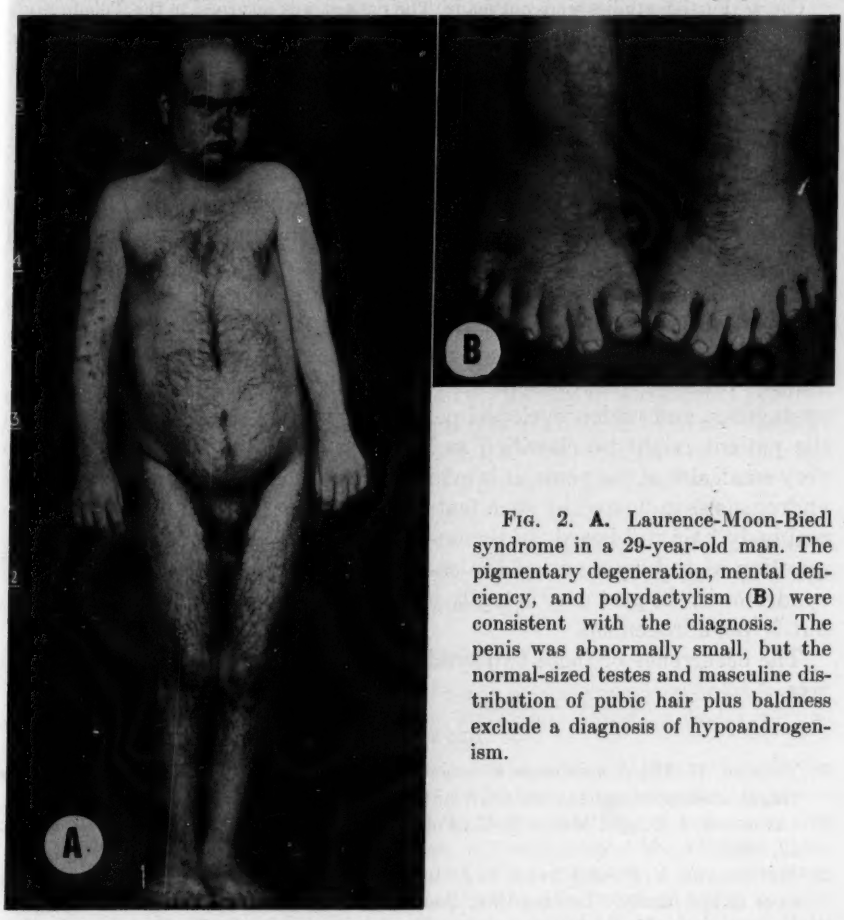

FIG. 2. **A.** Laurence-Moon-Biedl syndrome in a 29-year-old man. The pigmentary degeneration, mental deficiency, and polydactylism (**B**) were consistent with the diagnosis. The penis was abnormally small, but the normal-sized testes and masculine distribution of pubic hair plus baldness exclude a diagnosis of hypoandrogenism.

was bald, but facial hair was normal in amount and distribution. Body hair was unusually heavy in growth. The deep tendon reflexes were normal.

Laboratory findings. A testicular biopsy revealed fairly active spermatogenesis. The tubules were of approximately average size with many cells in the lumen. The germinal layer was of average thickness, and frequent spermatids and a fair number of spermatozoa were observed. An average amount of interstitial tissue of the usual density was present and there were frequent small masses of Leydig cells.

X-ray findings. The sella turcica measured 15 mm. in anteroposterior diameter, 13 mm. in depth, and was rounded. The fifth metatarsal bone of the left foot formed a Y with two toes extending from this bone, making 6 toes in all. The navicular bone was flattened and widened.

Course. Further studies were not made. The patient was returned to the Toledo State Hospital where he suddenly died in March 1949, nearly two years after examination. No autopsy was performed.

COMMENT

Both of these patients manifested many of the features of their respective syndromes. These were:

Turner's syndrome: Short stature, stocky habitus, webbed neck, cubitus valgus, wrist deformity, webbed toes, cardiac murmur, sparse pubic and axillary hair, sexual infantilism, amenorrhea, castrate vaginal smear, and high titer of urinary gonadotropins. Although morphologically female, the genetic sex was male.

Laurence-Moon-Biedl syndrome: Mental deficiency, psychotic behavior, ataxia, obesity, polydactylism, retinal pigmentation with blindness, nystagmus, and underdeveloped penis with normal-sized testes. Although the patient might be classified as having hypogenitalism because of the very small size of the penis, it is evident that there was no lack of testicular androgens as indicated by such features as normal testicular cytology, normal body hair and beard, and male-type baldness. These findings raise the question as to how many of the cases diagnosed as Laurence-Moon-Biedl syndrome in the past may actually have been cases of hypogenitalism without hypoandrogenism.

The occurrence of these two syndromes separately in siblings is distinctive.

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KLINEFELTER'S SYNDROME: REPORT OF A CASE STUDIED BY FLUOROHYDROCORTISONE SUPPRESSION OF ADRENAL FUNCTION

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KLINEFELTER'S syndrome now includes, according to the broadened concept of Heller and Nelson (1), all patients with azoospermia, small atrophic testes, clumped Leydig cells, hyalinized tubules, and increased urinary gonadotropins. The pathologic process involving the testes may be progressive, existing in different degrees at different times in a given individual. In addition to the originally described normally or subnormally virilized males (2), there is recognized a group of patients with varying degrees of eunuchoidism thought to depend on the age of the subject at onset of the disease. Gynecomastia and other physical abnormalities are variably present. In previous studies, a tendency to low 17-ketosteroid excretion has been observed, particularly in the more eunuchoid patients.

The following case is presented because the data suggest that a substantial portion of this eunuchoid patient's 17-ketosteroid excretion was of gonadal origin. It is also of interest that urinary corticoid excretion was elevated during an upper respiratory infection.

CASE REPORT

C.F. (Del. Hosp. 232307) was a 26-year-old man who was admitted to the hospital at the request of the Vocational Rehabilitation Bureau for study of his obesity (Fig. 1). The patient himself had no particular complaints.

History. He stated that he had been very obese since the age of 6 or 7 years. He believed that during his early childhood his head had been struck, resulting in unconsciousness. Prior to this episode he did not remember that he had been obese. His mother, at the time of the patient's admission, was moderately overweight; his father was quite thin although he was said at one time to have been heavy. The patient was an only child. He achieved the seventh grade in school and then quit. Ten or more years prior to admission he had been treated with injections of "male hormone" for about two years without any effect on his physical appearance. The details of this treatment were impossible to ascertain. Infection with mumps at any time was denied. No other significant illness or trauma was recalled.

At the time of admission there was no polyuria, polydipsia, or diarrhea. Appetite was admitted to be large and food consumption was two or three times normal. Some heat intolerance was present. Otherwise the review of systems revealed nothing abnormal.

Social sexual history. A lack of libido was denied. The patient stated he had had intercourse several times in the past, allegedly with ejaculation. He professed an interest in

females. He had many male friends and spent much time in masculine pursuits, such as playing pool with the boys.

Physical examination. The patient was an obese male who looked younger than his stated age of 26. His weight was 326 pounds. He had juvenile facies completely devoid of beard, and a female scalp line. His voice was high-pitched. There was no axillary hair and

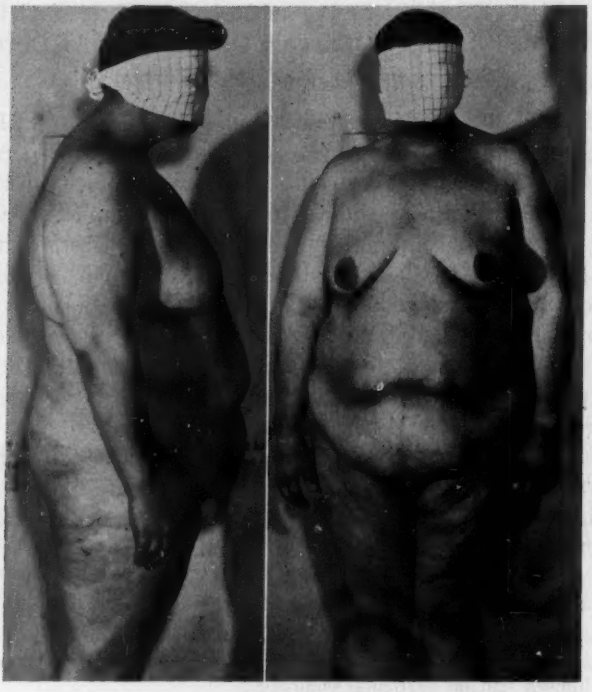

FIG. 1. Front and side views of Patient C.F.

very little pubic hair. The blood pressure was 140/90 mm. of Hg. Results of ophthalmoscopic and visual field examinations were normal. The breasts were enlarged and slightly pendulous. The excessive amount of adipose tissue made palpation of glandular elements difficult, but there seemed to be little, if any, glandular enlargement. The penis was small. Two soft scrotal testes were felt, measuring about 1.0 by 1.5 cm. The prostate was small. The distance from pubis to floor was 84 cm. (33 inches); from pubis to top of cranium, 174 cm. (68 inches); and the span was 188 cm. (74 inches).

Laboratory data. Preliminary data included a normal hemogram and a negative reaction in the test for syphilis. The urine was normal upon urinalysis; the specific gravity was 1.027. Roentgenograms of the skull demonstrated that the pituitary fossa was of normal shape and size and there were no intracranial calcifications. X-ray examination of both wrists showed complete maturation of the bones. Sperm analysis was attempted but the total volume of the "ejaculate" was only 0.5 cc. Complete azoos-

permia was evident in both a wet preparation and a stained smear. A testicular biopsy was reported by the pathologist (Dr. John W. Howard) as showing "complete atrophy of germinal epithelium, persistent Sertoli cells, fibrous thickening of the lamina propria and persistent Leydig cells arranged in conglomerate masses. Tubular hyalinization is not evident in this biopsy, suggesting the degeneration is not complete. Diagnosis: Sclerosing tubular degeneration (Klinefelter-Reifenstein-Albright Syndrome)."

In November 1955 and in February 1956 the urine was assayed for the presence of gonadotropin (FSH), 17-ketosteroids and estrogens (Table 1). There appeared to be a

TABLE 1. EFFECT OF 9-α-FLUOROHYDROCORTISONE ON URINARY EXCRETION OF HORMONES

	Control		After 6 days of 9-α-FHC	Control	Normal range
	Nov. 1955	Feb. 1956	Feb. 1956	Sept. 1956	
FSH (mouse units)	96	96–192	—	—	4–35
17-Ketosteroids (mg./24 hrs.)	23.1	15.4	9.0	13.6	9.0–22.0
Estrogens (mouse units)	27	13–27	13–27	—	13–27
Corticoids (mg./24 hrs.)	—	15.1	0	7.5	2.5– 9.0

mild discrepancy between the marked degree of clinical hypogonadism, suggesting a hypo-Leydig effect, and the upper-normal level of 17-ketosteroid excretion. Therefore, we sought to determine whether the 17-ketosteroids were being normally derived from both gonadal and adrenal sources, or whether they were entirely of adrenal origin. First, a baseline determination was made on the 24-hour urine collection of February 7, 1956 in order to recheck the normal 17-ketosteroid value of November 1955. Then on February 10 the patient was given 9-α-fluorohydrocortisone (5 mg. orally twice daily for six days) according to the technique for adrenal inhibition described by Goldfien *et al.* (3). On the fifth and sixth days urine was again collected for 17-ketosteroid assay. The results are tabulated in Table 1. Along with this study, previous FSH and estrogen assays were re-checked and in addition the urinary corticoids were measured. In September 1956, corticoids and 17-ketosteroids were measured again (Table 1).

DISCUSSION

The urinary hormonal pattern was consistent with that seen in the Klinefelter's syndrome—markedly elevated FSH, and normal levels of 17-ketosteroids and estrogens. In most patients with such striking hypogonadal signs the excretion of 17-ketosteroids has been in the low-normal or subnormal range rather than in the upper-normal range exhibited by this patient. It is apparent that administration of 9-α-fluorohydrocortisone induced inhibition of the adrenals, presumably by decreasing corticotropin stimulation. This effect was manifested by the disappearance of measurable corticoids from the urine, and by a marked fall (over one-third) in

ketosteroid excretion. Since corticoids are somewhat more easily inhibited than ketosteroids, this method is open to the criticism that the dosage of 9-α-fluorohydrocortisone was insufficient to achieve complete suppression. Probably there is not enough available experience with this technique to answer this criticism. However, the evidence from our study indicates that in this patient both adrenal and gonadal ketosteroids were present in normal proportions.

It will be noted that the baseline value for urinary corticoids (determined by the Porter-Silber method with glucuronidase hydrolysis) was 15.1 mg. per twenty-four hours. As this value is three or four times the average, and twice the upper limit of normal seen in our laboratory, it suggests adrenocortical hyperactivity. Paper chromatography, using essentially the Zaffaroni technique, demonstrated the excretion of α-ketolic steroids also to be about three times normal (4).

During the baseline collections in February 1956, the patient suffered from a slightly sore throat; on one occasion the temperature rose to 101.8° F. This, however, did not invalidate the purpose of the study—to determine whether or not there was a gonadal as well as an adrenal source of the 17-ketosteroids—since it would tend to exaggerate the relative importance of the adrenal source, should the ketosteroids be abnormally high during the baseline period. On the other hand, the important question raised by the increase in urinary corticoids is whether this hyperactivity was due to the stress of a mildly sore throat, or whether there was a true adrenal hypercorticism associated with the endocrinopathy characteristic of Klinefelter's syndrome.

In September 1956, the excretions of 17-ketosteroids and of corticoids were again determined. At this time no infection was present. The 17-ketosteroids were essentially unchanged but the urinary corticoids were half the former control value, suggesting that the abnormally elevated level found on February 7, 1956 was secondary to the stress of an acute infection at the time of collection, and that there was no hyperadrenocorticism under normal circumstances. Agnes Burt and associates (5) have presented a case of this syndrome and a review of 21 other cases examined at autopsy, including several with thyroid or adrenal abnormalities as demonstrated by anatomic and histochemical techniques. Their case had normal thyroid function, but it was suggested that Klinefelter's syndrome might be a multiglandular, rather than a pure gonadal disorder. Our case further emphasizes the need for more study of this question.

SUMMARY

A case of Klinefelter's syndrome is presented.

Suppression of adrenal steroids was attempted by administration of 9-

α-fluorohydrocortisone. The dose administered induced complete suppression of urinary corticoids and a distinct reduction (approximately one third) in 17-ketosteroids.

In this case it appears that the gonads as well as the adrenal cortex were a source of 17-ketosteroids.

Further study of adrenal function in cases of Klinefelter's syndrome seem indicated.

Acknowledgments

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TREATMENT OF EOSINOPHILIC GRANULOMA BY CORTICOTROPIN: REPORT OF 4 CASES WITH DISAPPEARANCE OF THE BONE LESIONS*

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IN 1952 Flosi *et al.* (1) reported the initial results of the use of corticotropin (ACTH) in the treatment of eosinophilic granuloma, showing recession of the tumoral mass as well as partial disappearance of the bone lesions.

The choice of corticotropin, which stimulates the secretion of the glucocorticoid hormones, was based on the known action of the latter upon circulating eosinophils (2).

Lackey *et al.* (3) and Mazzitello (4) observed beneficial effects from corticotropin therapy in cases in which eosinophilic granuloma was localized in the lung, and there have been a few other favorable reports (5, 6).

The object of this paper is to record the course of a case (observed for forty-three months) in which there was remission of the bone lesions, and to describe 3 later cases which confirmed the initial results.

CASE REPORTS

Case I

R.P.S. (H.C. 271887), a 5-year-old girl, was admitted on February 28, 1952.

About nine months prior to admission her father had noticed a swelling in the right occipital region which seemed to increase progressively. A similar swelling was next discovered in the right parietal region, and the patient started complaining of pain and suppuration in the right ear. Five months later it was noticed that her right eyeball was gradually beginning to bulge. She had neither fever nor any other abnormality, and her personal and family backgrounds were of no particular clinical interest.

Physical examination. She was 91 cm. in height and weighed 13.7 Kg. Her temperature was 36°C., pulse rate 80 per minute, respiratory rate 14 and, blood pressure 90/70 mm. Hg. In the right parieto-frontal region there was a circular boneless spot, and in the right occipital region a painless soft eggsized swelling. Right exophthalmos was also present. From the right acoustic duct projected a red, bloody, polyposis-like tumoral

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mass with an odorless secretion; it was painful to the touch. The rest of the examination revealed no hepatosplenomegaly, neurologic alterations, or any other abnormalities.

Laboratory data. Hemoglobin 8.3 Gm. per 100 ml; red blood cells 3,400,000 per cu. mm.; leukocytes 12,900 per cu. mm.; neutrophils 58 per cent; eosinophils 1 per cent; typical lymphocytes 37 per cent; monocytes 4 per cent. Serologic tests for syphilis—Wassermann (Kolmer's technic) and Kahn and Kline (cardiolipine antigen)—yielded negative results. The level of serum total protein was 7.9 Gm. per 100 ml; albumin 4.1 Gm., globulin 3.8 Gm., and A/G ratio 1:1 (Gornall-Bardawill-David). Tests of liver function yielded the following results: Takata-Ara, 122, 233, 221; formol-gel, negative; Weltmann test, clotting in tube No. 4; cephalin flocculation, negative; thymol turbidity, 3.9 McLagan units. The level of fasting blood sugar was 91 mg. per 100 ml. (Folin-Wu); total blood CO_2, 22 mEq. per L.; serum calcium, 9.2 mg. per 100 ml. (Clark-Collip); inorganic phosphorus, 5.8 mg. per 100 ml. (Fiske-SubbaRow); and phosphatase activity 16.1 King-Armstrong units. The urine was normal.

The patient remained in the ward for one hundred and ten days without any modification in her general condition. On June 18, 1952 a thorough curettage of the tumoral mass in the acoustic duct was performed. Pathologic examination of the tissue revealed numerous eosinophils, either isolated or in groups, alongside histiocytic cells, some of which had a xanthomatous appearance (Fig. 1A). A diagnosis of xanthomatous eosinophilic granuloma was made.

The tumoral mass, however, grew again rapidly and the suppuration became fetid. On two successive days in July the eosinophil count was 1,885 per cu. mm. and 762 per cu. mm.

On July 11 the administration of 100 mg. of corticotropin (ACTH[1]) daily was begun, separated into four intramuscular injections of 25 mg. each, every six hours. A diet with no salt was prescribed. During the first four days of the treatment the tumoral mass persisted, secreting a whitish and fetid fluid. The eosinophil count was 6 per cu. mm. on July 15 and 9 per cu. mm. on July 17. After five days the tumoral mass began to recede, and after ten days a speculum had become necessary for examination, although the secretion persisted. By the twelfth day the reduction in size was distinctly visible, and nearly half the duct had undergone atresia. The level of the circulating eosinophils had fallen to 0. This condition continued until July 28, when another biopsy was made. On this occasion there was more severe bleeding, and, for lack of a tumoral mass, parts of the wall of the duct where the remains of the tumor had infiltrated, were removed. Pathologic examination of the removed material revealed only histiocytic cells (some of which were xanthomatous) and no eosinophils (Fig. 1B). Next day the eosinophil count was 25 per cu. mm.

On August 4, corticotropin therapy was stopped, the total dose having been 2,400 mg. in the course of twenty-four days. The blood pressure was 95/60 mm. Hg; it had oscillated during the treatment, with an average level of 110/60 mm. When treatment was concluded, the only evident clinical alteration was edema of the face.

After omission of treatment there was an intensification of atresia of the acoustic duct and of the exophthalmos, but the tumoral mass did not reappear. On August 29 oral administration of cortisone was begun in a dosage of 100 mg. daily for eighteen days. After this therapy was discontinued, it was confirmed there was no modification of the exophthalmos.

The favorable change in the bone lesions under corticotropin therapy may be seen in Figure 2.

[1] Armour.

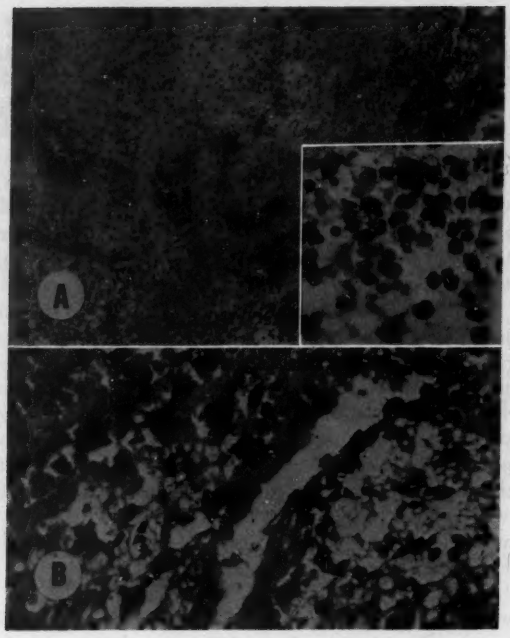

FIG. 1. (*Case I*). **A.** Low-power and high-power views of biopsy section from tumor in acoustic duct before treatment, showing reticular tissue infiltrated by numerous eosinophils (the majority of the dark cells), either isolated or in groups, alongside histiocytic cells, some of which appear xanthomatous. (H.E. stain.)

B. High-power view of section from tumor after treatment with corticotropin, showing only histiocytic cells (some xanthomatous) and no eosinophils. (H.E. stain.)

FIG. 2. (*Case I*). **A.** June 28, 1952. before treatment. Roentgenogram of skull showing extensive destructive lesions with irregular contours and no endosteal reaction in the fronto-parietal, inferior parietal, temporal and occipito-parietal regions.

B. Jan. 15, 1953, after treatment with corticotropin. The lytic lesions are more clearly outlined and there is endosteal reaction.

C. July 22, 1954. Disappearance of the lytic lesions. The newly formed tissue is slightly more transparent than normal skull bone.

Case II

U.N. (H.C. 317122), admitted on April 10, 1953, was a 6-year-old white boy, 110.5 cm. in height and weighing 17.6 Kg. His blood pressure was 100/60 mm. Hg.

About nine months prior to admission he complained of a painful elastic swelling around the left parietal region. The swelling was punctured, leaving a depression at the site. Four months later a similar swelling made its appearance in the right parietal region. Besides pain and some lack of appetite, the patient felt no other symptoms, and his general condition remained good. Two roundish depressions with irregular contours were found in the parietal region, forming a fairly symmetrical pattern. X-ray examination disclosed an area of osteolysis (Fig. 3A) underneath the depression. A biopsy of the right

FIG. 3. (*Case II*). **A.** April 10, 1953. Roentgenogram of the skull before treatment, showing extensive osteolytic lesion with irregular poorly defined outline and no endosteal reaction, in the left parasagittal region.

B. Feb. 18, 1954. After treatment with corticotropin. The lesion has decreased in size, is better defined, and there is repair enostosis.

C. Dec. 12, 1955. Only one small focus, with a well defined outline, remains.

parietal bone, along the line of the lesion, indicated the pathologic diagnosis of xanthomatous eosinophilic granuloma (Fig. 4, A and B.)

Laboratory data. Hemoglobin 12.2 Gm. per 100 ml. (76 per cent) erythrocytes 4,100,000 per cu. mm.; leukocytes 7,000 per cu. mm.; neutrophils 30 per cent; typical lymphocytes 55 per cent; leukocytoids 2 per cent; eosinophils 3 per cent; basophils 1 per cent; monocytes 8 per cent; and plasmocytes 1 per cent. The concentration of total serum protein was 7.3 Gm. per 100 ml.; albumin 5.6 Gm.; globulin 1.7 Gm.; cholesterol 109 mg. per 100 ml. (Sheftel); calcium 10.2 mg.; inorganic phosphorus 5.1 mg.; and alkaline phosphatase activity 13.5 Bodansky units. There was no Bence-Jones protein in the urine.

The treatment consisted of intramuscular administration of 60 mg. of corticotropin (ACTH) daily at the rate of 20 mg. every eight hours for a period of thirty-three days. Later the daily dose was reduced to 30 mg. (10 mg. every eight hours) for fourteen days. Then the treatment was interrupted, after the patient had received 2,400 mg. of hormone (from May 18 to July 3, 1953). Four days after the treatment was concluded a biopsy was made on bone from the left parietal region; it revealed regenerating areas in the bone tissues (Fig. 4C).

The beneficial effects of corticotropin in promoting regression of the bone lesions and restoration of normal bone tissue may be verified in the accompanying roentgenograms (Fig. 3).

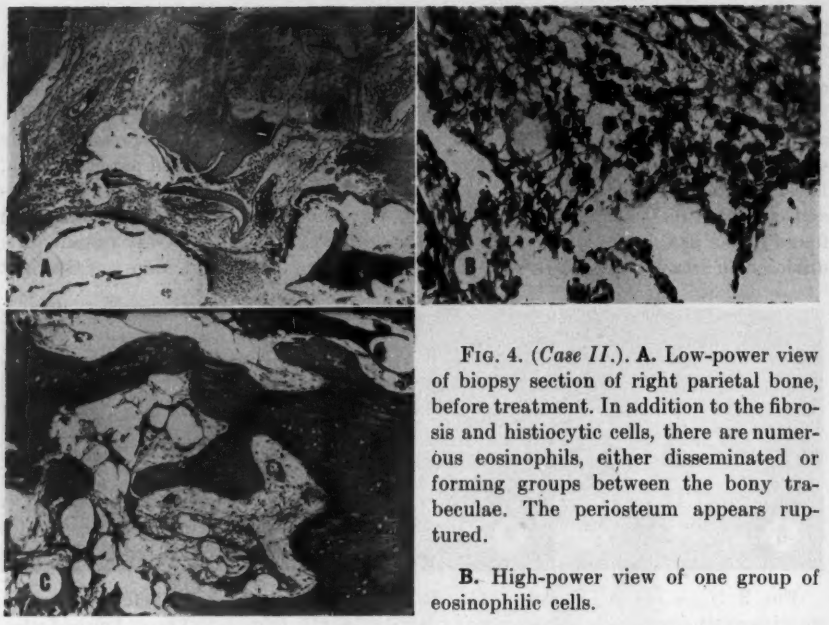

FIG. 4. (*Case II.*). **A.** Low-power view of biopsy section of right parietal bone, before treatment. In addition to the fibrosis and histiocytic cells, there are numerous eosinophils, either disseminated or forming groups between the bony trabeculae. The periosteum appears ruptured.

B. High-power view of one group of eosinophilic cells.

C. After treatment with corticotropin. The eosinophilic cells have disappeared and osseous tissue is regenerating with a continuous layer of periosteum. (All sections, H. & E. stain.)

Case III

J.E.P.M. (H.C. 34391), an 8-year-old white boy, was admitted on April 9, 1954. His weight was 26 Kg., height 124 cm., and blood pressure 104/60 mm. Hg.

The patient's illness had become evident some five months prior to admission, when he complained of toothache. A diagnosis of granuloma on the right upper jaw was made and a curettage performed. Pathologic examination of the material thus removed showed it to be an eosinophilic granuloma. About ten days before hospitalization the patient had presented right axillary adenopathy and a small swelling in the line of the second right rib.

On *physical examination* he was afebrile and in good general condition. He had no teeth on the right third of the upper jaw which connected with the right maxillary sinus, because of the curettage that had been performed. Over the second right rib, in the midclavicular line, there was a slightly painful swelling that felt less consistent than bone.

Laboratory data. Hemoglobin 10.4 Gm. per 100 ml.; erythrocytes 4,200,000 per cu. mm.; leukocytes 7,200 per cu. mm.; neutrophils 61 per cent; typical lymphocytes 28 per cent; eosinophils 2 per cent; basophils 1 per cent; and monocytes 5 per cent. The level of total serum protein was 7.2 Gm. per 100 ml; albumin 5.0 Gm.; globulin 2.2 Gm.; calcium 10 mg. per 100 ml.; inorganic phosphorus 4.5 mg. per 100 ml.; and alkaline phosphatase activity 5.9 Bodansky-units.

About two months following hospitalization a painful swelling appeared on the lower jaw in the area of the left canine, accompanied by loosening of that tooth and its neighbors.

Treatment by corticotropin (ACTH) was begun on June 14 with intramuscular doses of 25 mg. every six hours until a total dose of 2,400 mg. had been administered by July 7. During the first week of hormonal treatment the pain and the swelling disappeared from the lower jaw and the teeth became firm. The evolution of the bone lesions may be seen from the accompanying series of roentgenograms (Figs. 5 and 6).

Case IV

J.A.S. (H.C. 392213) was a 25-year-old single white army private.

He entered the Out-patient Service of the Department of Metabolism and Endocrinology on December 13, 1954 because of an intense pain for about five months in the back of his skull at the nape. He complained that he could not wear his army helmet, for the pain was such that he could not bear the slightest touch on his scalp. About fifteen days after the pain had started he had noticed a swelling in the region of the pain, so he went to a Medical Service, where an x-ray examination was made. Biopsy of the tumor revealed an eosinophilic granuloma, so he was sent to this Service.

On *physical examination* his various systems appeared normal. He was 160 cm. tall and weighed 56 Kg.; his pulse rate was 84 per minute, and his blood pressure 110/80 mm. Hg.

At the hospital, from January 10 to 25, 1955, the patient received corticotropin (ACTH) therapy as follows: five doses of 25 mg. dissolved in 500 ml. of dextrose solution (5 per cent) during eight hours, every other three days. When the treatment was concluded the pain had ceased and the swelling had subsided. He was submitted to x-ray examination before treatment and was checked periodically thereafter in order to permit following of the course of the bone lesion (Fig. 7).

COMMENTS

The work of Otani and Ehrlich (7) and of Lichtenstein and Jaffe (8) has permitted the recognition, within the group of reticuloendothelioses, of a special form of granuloma containing numerous eosinophils—the eosinophilic granuloma. It is characterized not only by the production of circumscribed bone lesions with no alteration of the general condition, but by a tendency to benign evolution.

Despite this recognized tendency to benign evolution, the beneficial effect of corticotropin therapy seems indubitable from the cases presented. Thus in Case I, after a period of one hundred and ten days under observation, not only was there no improvement, but curettage of the acoustical duct tumor showed a new tumoral mass which rapidly took the place of the one removed. However, when corticotropin was administered, the tumor gradually subsided, the exophthalmos receded, and x-ray studies showed a gradual remission of the bone lesions. In Cases II, III and IV, there was also gradual regression of the bone lesions after administration of corticotropin. In Case III, the "lamina dura" developed again (Fig. 6, C and D). In Cases II and III the disappearance of the bone lesions was not complete; there remained only a small boneless spot, probably corresponding to the area of the biopsy. Corticotropin was well tolerated, and there were no clinical or hormonal alterations which made its administration inadvisable.

FIG. 5. (*Case III*). **A.** April 4, 1954. Roentgenogram of thorax, before treatment, showing round osteolytic medullary lesion in the distal portion of the second rib.

B. Oct. 3, 1954, after treatment with corticotropin. The lesion is much smaller and noticeable only at the anterior end of the second rib.

C. Jan. 1, 1956, Region of the second rib, showing bone repair and complete disappearance of the lytic lesions.

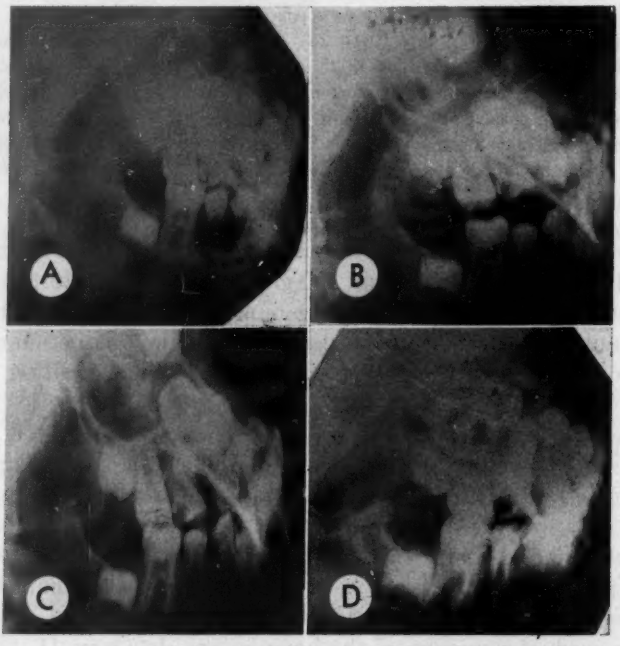

FIG. 6. (*Case III*). **A.** June 3, 1954. Roentgenogram of lower jaw, before treatment. There is focal destruction of the left alveolar border by an eosinophilic granuloma situated in the region corresponding to the left premolar teeth.

B. Oct. 13, 1954, after treatment with corticotropin, showing enostosis partially filling the destructive lesion.

C. Feb. 29, 1955. Complete repair of bone. The newly formed tissue is of rougher structure.

D. Jan. 20, 1956. Complete remission of the lesion, inducing reappearance of the lamina dura.

FIG. 7. (*Case IV.*) **A.** Feb. 23, 1955. Roentgenogram of skull shortly after corticotropin therapy started in January 1955, showing extensive focal destructive osteolytic lesion of poorly defined and irregular outline, situated in the mid-superior region of the squama occipitalis.

B. May 20, 1955. Reduction of the lytic lesion by enostosis around the focus.

C. Jan. 1, 1956. Almost complete bone repair.

In view of these results, corticotropin can be recommended for the treatment of eosinophilic granuloma.

SUMMARY

A report is presented of 4 cases of eosinophilic granuloma localized in bone, treated by administration of corticotropin (ACTH).

Recession of the bone lesions following therapy was demonstrated in all cases by roentgenograms, and in 2 cases by histopathologic findings.

Corticotropin is an effective form of treatment for eosinophilic granuloma.

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Letters to the Editor

A CASE OF TRUE HERMAPHRODISM: POSSIBLE RELATIONSHIP TO KLINEFELTER'S SYNDROME

To the Editor

The finding that some cases of the syndrome characterized by gynecomastia, aspermatogenesis without a-leydigism, and increased excretion of follicle-stimulating hormone (Klinefelter's syndrome) have female-type chromatin patterns (1–5) has led to the speculation that these patients represent a form of gonadal dysgenesis. Grumbach *et al.* (6) have pointed out that these individuals represent the ultimate in sex reversal, and are in fact, a type of true hermaphrodism. Furthermore, they suggest that in some instances of classic true hermaphrodism in which both testicular and ovarian elements are present, the gonadal dysgenesis is actually less complete than in cases of Klinefelter's syndrome with female genetic sex. We have recently seen an infant with hermaphrodism, who apparently represents a form of marked sex reversal but to a degree less than the Klinefelter "male."

The patient was an 8-month-old white infant in whom an abnormality of the external genitalia had been noted at birth. The child had been considered a male because gonads presumed to be testes had been visible in the scrotum since birth. The parents were both well and the mother had no difficulty during pregnancy other than moderate nausea and vomiting throughout. No special medications had been taken. Labor and delivery were reported as normal. Growth and development of the infant had been normal. There was no outstanding history of illnesses.

The height and weight were normal for the age, and the other findings on physical examination were normal except for the genitalia (Fig. 1). There was a phallic structure approximately 2 cm. in length bound down ventrally by chordee. Below the phallus and between loose scrotal-appearing folds there was a cleft with a small opening from which urine was emitted. In the scrotal folds gonad-like structures were palpated, each measuring 2.0×0.8 cm. The initial impression was male pseudohermaphrodism. However, a smear of the buccal mucosa demonstrated a "female-type chromatin pattern," which prompted further studies.

When cystoscopy was performed it was noted that on the posterior wall of the urogenital sinus, inferior to the urethral orifice, there was a small opening which admitted a catheter. Roentgenograms made after radiopaque material had been introduced through the catheter revealed a small blind pouch beneath the bladder.

An exploratory laparotomy was performed, but there was no evidence of internal genitalia. When the contents of the scrotum were exposed (Fig. 2), there appeared to be two separate gonads on either side. These consisted of ovoid bodies arranged end-to-end in a common thin fibrous sheath, but clearly distinct from each other. The small superior

structures, which measured approximately 0.6 cm., were light tan in color and had a tense, firm consistency. The inferior structures measured 1.8×1.2 cm. and had a moderately firm, smooth consistency with a darker bluish-grey color. Intimately attached to each of the larger gonads was a dense white cord, presumably an epididymis, which continued superiorly as a vas deferens. Another separate cord-like structure, approximately 3.5 cm. in length, was curled above and loosely attached to each smaller gonad. This cord was widened at the end, and resembled the fimbriated portion of a Fallopian tube.

FIG. 1. External genitalia.

Biopsy of both superior gonads revealed numerous well formed primordial follicles embedded within a dense stroma (Fig. 3). Examination of the larger inferior gonads showed closely arranged tubular structures embedded in a loosely cellular stroma (Fig. 4). The tubules rarely had well defined lumens, and some were filled with a finely granular eosinophilic material. In many of the cells of the tubules there was the peripheral clumping of nuclear chromatin characteristic of the female chromatin pattern. There was no evidence of spermatogonia or Leydig cells.

It would appear that in this infant, because of genetic or unknown factors, there had been a differentiation of the indifferent fetal gonad into histologically well developed and separate testicular and ovarian elements. The testicular elements seemed to have exerted the greatest influence on

FIG. 2. Contents of each scrotal fold.

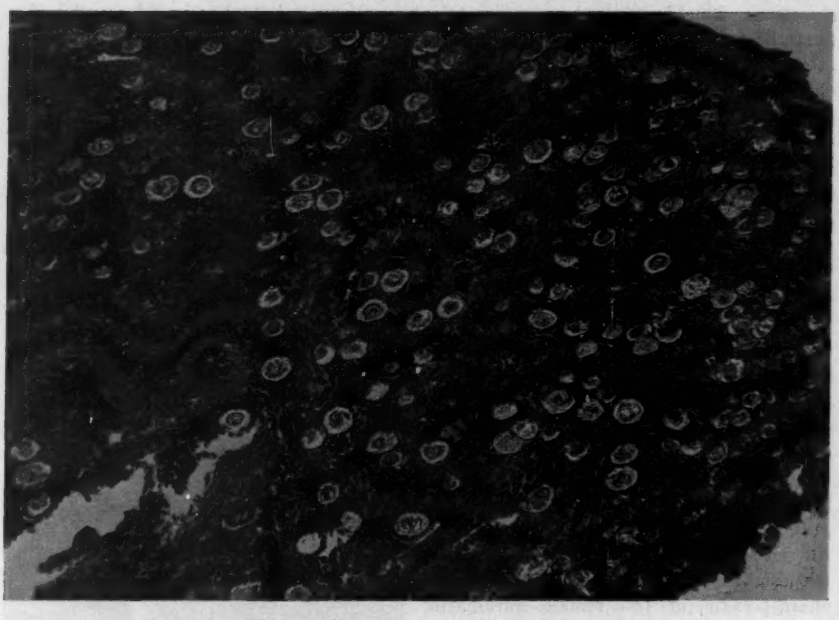

FIG. 3. Biopsy of superior gonad.

FIG. 4. Biopsy of inferior gonad.

sexual development, since only vestiges of the Müllerian system remained. Furthermore, the ovarian structures, and certainly what appeared to be Fallopian tubes, had accompanied the descent of the testes into the scrotum. It is difficult to say what influence, if any, the ovarian structures had on the persistence of the urogenital sinus and the hermaphroditic appearance of the external genitalia. It is tempting to postulate that if there had been complete dysgenesis of the cortical portion of the indifferent gonad, the result would have been a Klinefelter "male" without evidence of the Müllerian system.

Because the decision has been made to raise this infant as a male, it is contemplated that the ovarian structures and what appear to be Fallopian tubes, will be removed. Of particular interest will be the post-puberal examination of the testes as well as a study of their hormonal secretions.

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EFFECT OF ANXIETY UPON ALDOSTERONE EXCRETION IN MAN

TO THE EDITOR:

In the course of measuring aldosterone in healthy persons under a variety of conditions, occasionally high values were found which could be associated with stressful situations. These preliminary results were reported in

TABLE 1

Subject	Steroid	Urinary excretion					Reaction to stress
		Control period		During examinations			
		Day 1	Day 2	Day 1	Day 2	Day 3	
J.G.	Aldosterone (μg./24 hrs.)	2.4	3.4	5.1	3.5	—	Calm
	17-OH-CS (mg./24 hrs.)	7.8	8.2	4.6	4.2	—	
C.S.	*	7.4	—	8.1	—	—	Calm
		5.2	—	4.8	—	—	
W.S.		3.9	—	2.6	—	—	Calm
		4.6	—	8.0	—	—	
T.Mc.		13.4	1.7	6.1	6.8	6.5	Anxiety 4+ on 1st day of urine collection
		10.0	6.6	6.4	5.8	4.6	in control period. Calm during exams.
N.D.		11.4	—	2.2	—	—	Anxiety 3+ in control period. Calm during
		6.4	—	4.2	—	—	exams.
J.N.		1.0	1.1	2.4	1.1	6.8	Anxiety 1+ to 2+ during exams.
		6.2	4.6	9.6	4.4	6.2	
S.L.		1.9	1.2	4.2	6.6	6.0	Anxiety 1+ to 2+ during exams.
		5.8	6.4	8.6	7.4	6.6	
N.W.		2.2	—	6.7	—	—	Anxiety 2+ during exams.
		5.6	—	7.6	—	—	
O.R.		2.0	3.5	7.0	6.0	—	Anxiety 2+ during exams.
		4.4	5.0	5.1	4.7	—	
G.S.		4.0	5.6	5.5	6.5	9.1	Anxiety 2+ during exams.
		6.2	4.6	4.8	6.0	9.2	
R.P.		1.5	4.5	1.1	2.4	7.2	Tension increased during exams., from 1+
		5.0	6.8	2.6	3.0	5.8	to 3+
R.M.		2.2	1.5	8.6	6.5	—	Anxiety 3+ during exams.
		4.4	4.8	4.3	4.8	—	
P.S.		3.4	2.1	2.7	2.4	7.6	Anxiety 4+ during exams.
		6.4	4.4	17.5	10.0	8.2	
E.M.		5.8	—	11.2	—	—	Anxiety 4+ during exams.
		3.6	—	5.0	—	—	
R.H.		1.3	<1	3.7	8.2	18.0	Anxiety 3+ to 4+ during exams.
		6.0	5.6	11.7	16.3	17.0	
D.B.		4.0	7.1	4.1	13.3	14.3	Very tense and anxious, 4+ during exams.
		7.8	6.8	17.7	7.5	10.5	
W.L.		7.9	—	13.0	—	—	Anxiety 4+ during exams.
		5.5	—	4.8	—	—	
M.L.		2.0	5.0	20.0	—	—	Anxiety 4+ during exams.
		2.0	3.0	9.0	—	—	

* The same two determinations were made in each subject.

1956 (1). Lamson *et al.* (2) have also observed higher than normal amounts of aldosterone in the urine of nonpsychotic subjects with symptoms of anxiety. In order to study this subject further we have investigated the effect of an emotional stress, such an anxiety, in 18 students undergoing examinations. Urine was collected from the students over a period of one to three days approximately one month before the examinations. Similar collections were made during the period of examinations. Aldosterone was measured by means of a bioassay (3) and 17-hydroxycorticosteroids by a chemical procedure (4). The students were interviewed separately and the degree of anxiety experienced before and during the examinations was graded from 1+ to 4+. This was based upon several factors such as apprehension and inability to concentrate, sleep and eat. There was considerable variation in the degree of stress that the students experienced and it was evident that only an approximation could be made. Two of the students stated that they were very apprehensive prior to the examinations but had no concern during the actual examination period. The results are shown in Table 1.

In the students who claimed that they were not emotionally disturbed by the examinations, there was little change in aldosterone excretion. In those who graded their anxiety as moderate, there was a rise in aldosterone which did not, however, exceed the upper limit of normal range. In 5 out of 6 students who claimed that they were unduly stressed, there was a significant rise in the aldosterone excretion. The average values for aldosterone excretion in these three groups of students during the period of examination were 4.8, 5.5 and 10.0 μg. per twenty-four hours, respectively. The 2 students who were extremely anxious before the examinations had high aldosterone excretions at that time. In several of the students the excretion of 17-hydroxycorticosteroids also increased during the period of stress.

There is ample evidence that emotional stress can activate the adrenal cortex in man. Increases in 17-ketosteroid (5) and 17-hydroxycorticosteroid excretion (6), as well as a rise in the 17-hydroxycorticosteroid level in peripheral blood (7) have been reported during acute anxiety. The present studies indicate that in certain persons acute anxiety is also associated with a rise in aldosterone excretion.

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Acknowledgments

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THYROIDAL UPTAKE OF I^{131} AFTER IOPANOIC ACID (TELEPAQUE) IN 74 SUBJECTS

TO THE EDITOR:

Although suppression of I^{131} uptake immediately after the administration of gall-bladder contrast media is well recognized, the duration of interference is rather uncertain. Estimates for euthyroid subjects vary from one month (1, 2) or six months (3), to one year (4). Except for 3 reports (1, 2, 5) on groups of 4 to 7 patients subjected to control measurements before receiving the medium, reference values for normal uptake have not been obtained in the specific individuals under study. The present communication is concerned with a group of 74 euthyroid male subjects in whom uptake was measured both before and after administration of iopanoic acid (Telepaque).

METHODS

The group comprised 63 hospitalized patients and 11 staff employees who had received no iodine-containing medication within the past year. Following a routine 24-hour estimation of thyroidal I^{131} uptake using a conventional scintillation counter and an oral dose of 10 to 50 microcuries

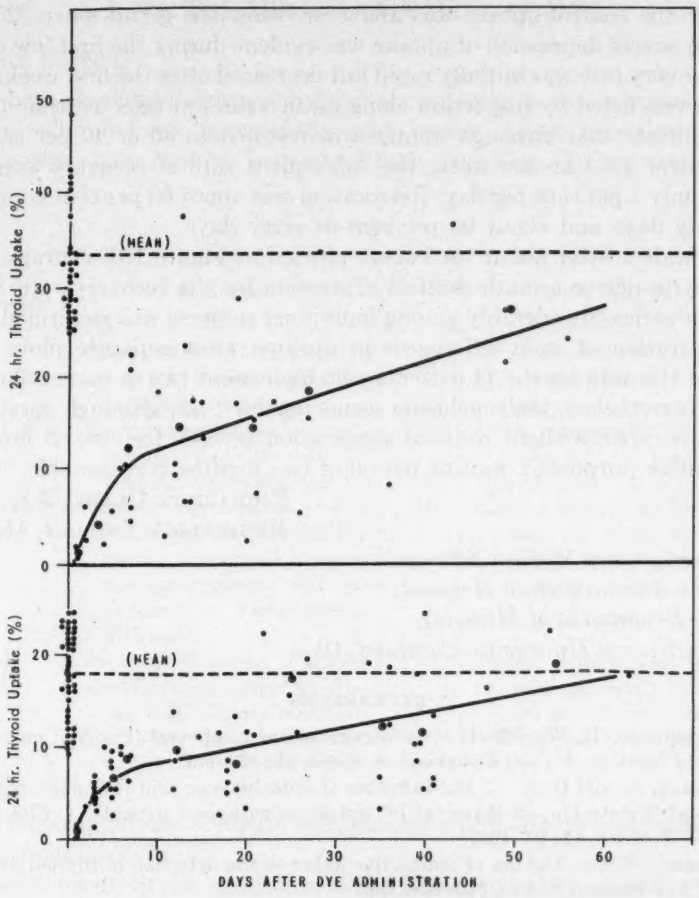

FIG. 1. Uptake of I^{131} by the thyroid over a period of sixty days following administration of Telepaque.

of NaI^{131}, a 3-Gm. dose of Telepaque was administered orally. Subsequent tracer studies were performed at random time-intervals ranging from one to sixty-three days after administration of the dye. In most cases a single follow-up tracer study was performed, but in 14 cases there were two or three such tests.

RESULTS

In order to achieve greater homogeneity, the thyroidal uptake values depicted in Figure 1 were separated into two groups selected according to

whether the control uptake was above or below the grand mean (25 per cent). A severe depression of uptake was evident during the first few days. The recovery rate was initially rapid but decreased after the first week. The two curves, fitted by inspection along mean values at selected time-intervals, indicate that although uptake was restored to 30 or 40 per cent of the control level at one week, the subsequent rate of recovery approximated only 1 per cent per day. Restoration was about 60 per cent complete at thirty days and about 90 per cent at sixty days.

The wide scatter about the curves plotted in Figure 1 discourages any attempt to derive a mathematical expression for the recovery rate. That the rate varies considerably among individual subjects was confirmed by a demonstration of wide differences in upslope when separate plots were made of the data for the 14 patients who underwent two or more follow-up tests. Nevertheless, the conclusion seems tenable that, although partial recovery is rapid, a slight residual suppression persists for several months. For routine purposes a waiting period of two months is reasonable.

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A NEW HYPOGLYCEMIC AGENT

To the Editor:

N′-beta-phenethylformamidinyliminourea, DBI, a new synthetic substance[1] administered parenterally and orally causes hypoglycemia in guinea pigs, rats, rabbits, cats and Rhesus monkeys. It can also reduce

Table 1

Type of diabetes	Definition	No. of cases	Result with DBI
Mild	Requirement for insulin equivocal and largely dependent on dietary discretion	13	Urine consistently sugar-free; fasting glycemia <150 mg./100 ml.
Moderate	Requirement for insulin established but <20 units of long-lasting insulin/day	23	Urine consistently sugar-free; glycemia <150 mg./100 ml. in all cases
Moderately severe	Insulin requirement <20 units/day, but ketosis not readily developed despite marked glycosuria and hyperglycemia when insulin withheld	15	Control without insulin equivalent to that previously attained with insulin
Severe, labile	Rapid development of ketosis when required insulin dosage only slightly modified, or when stress increased	3	Insulin requirement reduced 25–50 per cent; control as good as with previous insulin dosage (in 1 case better)

blood sugar in alloxan-diabetic rats, rabbits and monkeys and maintain it at a normal level (1).

Species sensitivity is variable and the Rhesus monkey is most sensitive to the drug. In this animal DBI was effective at a level of 10 mg. per Kg. of body weight. Glucose tolerance studies in diabetic and nondiabetic patients, with and without the drug, indicated hypoglycemic activity at less than 2 mg. per Kg. (2).

A clinical study which included 54 diabetic patients in all age groups and of all degrees of severity showed an effect on glycemia and glycosuria in all who tolerated the drug in sufficient dosage (Table 1). The longest period of treatment of any patient is now nine months.

An elderly patient previously taking 100 units of insulin per day and a 44-year-old patient who had diabetes of seventeen years' duration requir-

[1] The drug DBI, and the results of the animal studies were made available by Dr. Louis Freedman, U. S. Vitamin Corporation, New York, N. Y.

ing 90 units of insulin per day have had no insulin for more than four weeks while receiving 150 mg. of DBI per day. Diabetic control was as good as that previously achieved with insulin. Middle-aged and elderly patients requiring up to 50 units of insulin per day are now being controlled with 75 mg. of DBI per day without insulin.

Three young patients with severe diabetes, prone to ketosis with slight modification of insulin dosage, are now better controlled with a 25–50 per cent reduction in insulin dosage plus 150 mg. of DBI per day. All of these patients formerly required more than 50 units of long-acting insulin daily.

In none of the patients has any evidence of hematologic, hepatic, electrolytic or renal disturbance been observed. Side-effects have been limited to some nausea and, in a few cases, vomiting, which usually can be controlled by reducing the dosage.

DBI is an active hypoglycemic agent. Its activity is evident in the young patient with severe labile diabetes mellitus as well as in those with moderately severe and mild diabetes, without manifestations of toxicity.

There is at present a limitation of dosage due to nausea and sometimes vomiting. The results here presented lend encouragement to wider investigation.

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The Endocrine Society

THE 1958 ANNUAL MEETING

The Fortieth Annual Meeting of The Endocrine Society will be held in the St. Francis Hotel, San Francisco, California, Thursday, Friday and Saturday, *June 19, 20 and 21, 1958.*

THE 1958 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955, Dr. Frederick L. Hisaw in 1956, and Dr. Joseph C. Aub in 1957.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

NINTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE MEDICAL COLLEGE OF GEORGIA

Augusta, Georgia, October 21-25, 1957

The faculty will consist of 24 eminent clinicians and investigators from various parts of the country in the fields of endocrinology and metabolism. The program will cover the various endocrinopathies, with emphasis on the clinical aspects, demonstration of laboratory tests, presentations of cases, and question-and-answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialties who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the Assembly.

For further information concerning the program and registration, write to *Dr. Robert B. Greenblatt, Department of Endocrinology, Medical College of Georgia, Augusta, Georgia.* Registration is limited to 100; the tuition fee is $100.00. Rooms will be reserved for the students and faculty at the Bon Air Hotel. Residents and fellows will be admitted for $35.00.

(*For Faculty and Program, see June issue*)

NOTICE TO READERS

It has been decided by the Council of The Endocrine Society that, because of the excessive expense, the abstracts of papers presented or read by title at the Annual Meeting will not be published in this journal.

AMERICAN SOCIETY FOR STUDY OF STERILITY GRANTS-IN-AID

The Ortho and Carl G. Hartman grants-in-aid of the American Society for the Study of Sterility, each of $500, are available for 1958. Applications for these two grants-in-aid should be sent to the Chairman of the Research Correlating Committee of the American Society for the Study of Sterility, Dr. S. Leon Israel, 2116 Spruce Street, Philadelphia 3, Pa., *by November 30, 1957*. No application will be considered by the Committee unless it is accompanied by 3 copies of a brief outline of the research project for which the grant-in-aid of $500 is being sought.

NEW YORK ACADEMY OF MEDICINE— POSTGRADUATE WEEK

The 1957 Postgraduate Week program is entitled "Research Contributions to Clinical Practice," and will be conducted over a period of five days, *October 7–11, 1957*, with daily evening lectures, afternoon panel meetings and a scientific exhibit. It will replace the Graduate Fortnight of former years. All inquiries regarding registration should be addressed to: Secretary, Postgraduate Week, The New York Academy of Medicine, 2 East 103 Street, New York 29, N. Y.